

**USADA and Floyd Landis**  
**AAA No. 30 190 00847 06**

**United States Anti-Doping Agency's List of Exhibits\***

<b>Tab</b>	<b>Exhibit</b>	<b>Bates Numbers</b>
1.	UCI Rules	
2.	Bylaws of the United States Olympic Committee	
3.	USADA Protocol for Olympic Movement Testing	
4.	World Anti-Doping Code	
5.	WADA 2006 Prohibited List	
6.	International Standard for Testing	
7.	Olympic Movement Anti-Doping Code	
8.	International Standard for Laboratories	
9.	TD2004EAAS	
10.	TD2004MRPL	
11.	TD2003LDOC	
12.	TD2003IDCR	
13.	USADA v. Hartman	
14.	Susin v. FINA	
15.	IAAF v. CBAt and Dos Santos	
16.	WADA v. Wium	
17.	IAAF v. Czech Athletic Federation and Roman Zubek	
18.	UCI v. Moller	

\*USADA reserves the right to supplement these exhibits with any of the following materials: documents pertaining to the further analysis of Mr. Landis's other samples; documents related to discovery requests to Mr. Landis that are still pending; and any additional rebuttal exhibits necessary after review of Respondent's brief and exhibits, including any relevant demonstrative exhibits.

19.	UCI v. Bakker	
20.	UCI v. Skelde	
21.	UCI v. Danmarks	
22.	UCI v. Landaluce (English and French translations)	
23.	ISO/IEC 17025 (1999) and (2005)	USADA 1250-1318
24.	A Sample Laboratory Documentation Package	USADA 0001-0223
25.	B Sample Laboratory Documentation Package	USADA 0224-0370
26.	LNDD Documents	LNDD 0001-0519
27.	Respondent's Doping Control Form (CPLD Copy)	Produced by Landis
27a.	CPLD Copies of other riders doping control forms received by CPLD on July 27	
27b.	CPLD Copies of other riders doping control forms received by CPLD on July 28	
28.	Respondent's UCI License Application	USADA 1250-1252
29.	Longitudinal Study of Respondent's T/E Ratio	
30.	Longitudinal Study of Respondent's T/E Ratio Adjusted for Specific Gravity	
31.	Photograph of the type of cooler and metal band used to transport Respondent's sample	
32.	Color Photographs of LNDD operating the Isoprime instrument at 5E-6 mbar	
32a.	LNDD's Response to alleged IRMS Documentation Errors	
32b.	Documentation tracking sample 995474 CG-MS	
33.	Excerpt from Dr. Arnie Baker's PowerPoint Presentation	
34.	Cologne WADA Research Project Progress Report March 2007	
34a.	Cologne PowerPoint	

35.	UCLA Final Report on WADA Grant - Pharmacokinetics of Pharmaceutical Testosterone and TE in Subjects with Low and High Baseline TEs: The assessment of the ethnic differences and of the sensitivity of various carbon isotope ratio methods relative to TE, Don H. Catlin	
36.	UCLA Report to USADA on research subject IVT-1	
37.	Illustrations of Steroid Metabolism	
38.	Figures 1-21 from USADA Brief	
39.	<p>Press Statements by Respondent</p> <ul style="list-style-type: none"> <li>• Sal Ruibal, <i>Synthetic testosterone found in Landis' 'B' sample; Tour winner likely to lose title</i>, USA TODAY, accessed Feb. 8, 2007, <a href="http://www.usatoday.com/sports/cycling/2006-08-04-landis-doping-test_x.htm">http://www.usatoday.com/sports/cycling/2006-08-04-landis-doping-test_x.htm</a></li> <li>• Associated Press, <i>Tour de France Champ Floyd Landis Requests Test of Backup Urine Sample in Doping Flap</i>, Fox News.com, Jul. 31, 2006, <a href="http://www.foxnews.com/printer_friendly_story/0,3566,206449,00.html">http://www.foxnews.com/printer_friendly_story/0,3566,206449,00.html</a></li> <li>• Sal Ruibal, <i>Landis fires back at cycling hierachy [sic]</i>, USA TODAY, accessed Feb. 8, 2007, <a href="http://usatoday.printthis.clickability.com/pt/cpt?action=cpt&amp;title=USATODAY.com+-+Lan...">http://usatoday.printthis.clickability.com/pt/cpt?action=cpt&amp;title=USATODAY.com+-+Lan...</a></li> </ul>	

40.	Additional Science Articles	
	<ul style="list-style-type: none"><li>• <i>Validity of urine samples :microbial degradation. Recent advances in doping analysis</i>, Ayotte C., Charlebois, A., Lapointe, S., Barriault D. and Sylvestre M.</li></ul>	USADA 1154-1164
	<ul style="list-style-type: none"><li>• <i>Variability of T/E ratios in Athletes</i>, Baenziger J. and L. Bowers</li></ul>	USADA 1165-1170
	<ul style="list-style-type: none"><li>• <i>Issues in detecting abuse of xenobiotic anabolic steroids and testosterone by analysis of athlete's urine</i>, Catlin D. H., C. K. Hatton and S. H. Starcevic</li></ul>	USADA 1171-1179
	<ul style="list-style-type: none"><li>• <i>Long-term administration of testosterone enanthate to normal men: alterations of the urinary profile of androgen metabolites potentially useful for detection of testosterone misuse in sport</i>, Dehennin L. and A. M. Masumoto</li></ul>	USADA 1180-1190
	<ul style="list-style-type: none"><li>• <i>Evaluation of longitudinal studies, the determination of subject based reference ranges of the T/E ratio</i>, Donike M., S. Rauth, U. Mareck-Engelke, H. Geyer and R. Nitschke</li></ul>	USADA 1191-1194
	<ul style="list-style-type: none"><li>• <i>Stability of steroid profile (2) : excretion rates from morning urines</i>, Mareck-Engelke U., H. Geyer and M. Donike</li></ul>	USADA 1195-1197
	<ul style="list-style-type: none"><li>• <i>Improved method of detection of testosterone abuse by gas chromatography /combustion/ isotope ratio mass spectrometry analysis of urinary steroids</i>, Aguilera R., M. Becchi, H. Casabianca, C.K. Hatton, D. H. Catlin and B. Starcevic</li></ul>	USADA 1198-1205
	<ul style="list-style-type: none"><li>• <i>GC/C/IRMS and GC/MS in "Natural" Steroids Testing</i>, Ayotte C., D. Goudreault, A. Lajeunesse, M. Cl��roux, Y. Richard, A. Charlebois, J. -P. Couture and A. Fakirian</li></ul>	USADA 1206-1211
	<ul style="list-style-type: none"><li>• <i>Kinetic isotope effects during metabolism of delta-4-steroids</i>, Flenker U. and Schanzer W</li></ul>	USADA 1212-1215
	<ul style="list-style-type: none"><li>• <i>Validity of carbon isotope ration measurements for decomposed urine samples</i>, R. Taylor, A. Cawley, R. Kazlauskas, G. Trout &amp; A. George</li></ul>	USADA 1216-1218
	<ul style="list-style-type: none"><li>• <i>Developments in Sports Drug Testing</i>; Trout, G., J. Rogerson; A. Cawley and C. Alma</li></ul>	USADA 1219-1224



	<ul style="list-style-type: none"> <li>• <i>Performance Characteristics of a Carbon Isotope Ratio Method for Detecting Doping with Testosterone Based on urine Diols: Controls and Athletes with Elevated Testosterone/Epitestosterone Ratios</i>, Aguilera, R. T. Chapman, B. Starcevic, C. Hatton, D. Catlin</li> </ul>	USADA 1225-1233
	<ul style="list-style-type: none"> <li>• <i>Improved Detection of Sugar Addition to Maple Syrup Using Malic Acid as Internal Standard and in <math>^{13}\text{C}</math> Isotope Ratio Mass Spectrometry (IRMS)</i>, Tremblay, P. and R. Paquin</li> </ul>	USADA 1234-1240
	<ul style="list-style-type: none"> <li>• <i>Confirming Testosterone administration by isotope ratio mass spectrometric analysis of urinary androstenediols</i>, Shackleton, C., A. Phillips, T. Chang and Y. Li</li> </ul>	USADA 1241-1249

41.	USADA Documents	USADA 0371-0475
42.	USADA Documents	USADA 0476-0731
43.	USADA Documents	USADA 0732-0970
44.	USADA Documents	USADA 0971-1002
45.	USADA Documents	USADA 1003-1007
46.	USADA Documents	USADA 1008-1137
47.	USADA Documents	USADA 1138-1153
48.	Excerpt from Operating Manual for IsoPrime (Pg 103)	
49.	WADA Documents	WADA 0001-0143

**TAB 46**

1

Final Report on USADA Grant entitled:  
Improving Urine Testing for Endogenous Steroids by Isotope Ratio  
Mass Spectrometry

Dates covered: April 1, 2003 - Feb 28, 2005  
Don H. Catlin, M.D.

**Specific Aim I:** To determine the feasibility and rationale of lowering the IOC cutoff for reporting T/E cases from 6 to 4.

**Hypothesis:** To the extent that athletes are using testosterone, epitestosterone, or both, and successful in avoiding detection by the IOC criteria for T/E (>6) and epitestosterone (>200ng/mL), they are likely to have unusually high (T/E = 5-6) or low (<0.3) urinary T/E ratios. Determining the  $\delta^{13}\text{C}$  values of urinary steroids on such samples will define the extent of exogenous steroid use in urine supplied by these athletes.

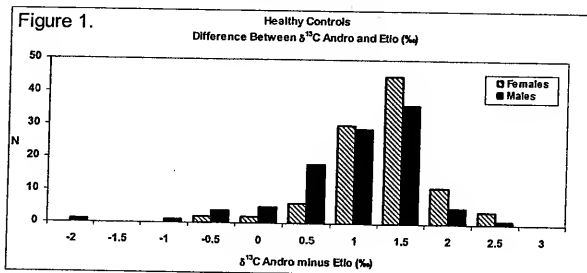
**1) What progress has been made?**

The  $\delta^{13}\text{C}$  values of androsterone and etiocholanolone have been measured in 100 male and 100 female control subjects. The data are summarized in Table 1 ( $\delta^{13}\text{C}$  values are expressed in ‰ units).

	Males		Females	
	Etio	Andro	Etio	Andro
N	100	100	100	100
Min	-25.2	-24.8	-25.7	-24.8
Max	-20.0	-19.0	-20.3	-20.4
Mean	-23.1	-22.3	-23.5	-22.5
SD	1.0	0.9	0.9	0.8
Mean-2SD	-25.1	-24.0	-25.3	-24.1

**Main Finding 1** The  $\delta^{13}\text{C}$  values for both androsterone and etiocholanolone are normally distributed for the female population as are the  $\delta^{13}\text{C}$  values for etiocholanolone in the male population. The  $\delta^{13}\text{C}$  values for androsterone in males are not normal ( $A=1.138$ ,  $p=0.005$ ) with a standardized kurtosis of 2.59. After removal of two  $\delta^{13}\text{C}$  values at each extreme (-19.012, -19.717, -24.806 and -24.782) by successive applications of Grubb's test using  $p<0.05$ , the null hypothesis of a normal distribution is no longer rejected ( $A=0.327$ ,  $p=0.105$ ). The extreme values in question do not correspond to extreme values of androsterone concentration and cannot be traced to measurement error. It is possible that the outliers are the result of diets that are extreme in  $\delta^{13}\text{C}$  values. A single outlier at -20.259 similarly identified among the  $\delta^{13}\text{C}$  values for etiocholanolone in females may have the same origin. Correlation coefficients for  $\delta^{13}\text{C}$  values of androsterone and etiocholanolone are 0.74 and 0.80 for males and females, respectively. Mean differences in  $\delta^{13}\text{C}$  values for androsterone and etiocholanolone are significant for both males and females at 0.74 and 1.08, respectively (Figure 1). The observation that the etiocholanolone

$\delta^{13}\text{C}$  value is more negative than the androsterone value in most urine samples has also been made in other WADA-accredited labs.



**Main Finding 2** We have completed the screening of 500 athlete samples by their  $\delta^{13}\text{C}$  values of androsterone and etiocholanolone. The data are summarized in Table 2.

Table 2.

	Males Low T/E		Males T/E~1		Males High T/E *	
	Etio	Andro	Etio	Andro	Etio	Andro
N	48	48	204	204	202	202
Min	-24.8	-23.9	-25.9	-24.0	-26.3	-25.8
Max	-21.7	-20.0	-20.1	-19.2	-19.7	-18.8
Mean	-23.0	-21.8	-22.4	-21.2	-22.6	-21.4
SD	0.8	0.9	1.0	0.9	1.2	1.1
* not including 1 case with TE=6.0						
	Females Low T/E		Females T/E~1		Females High T/E	
	Etio	Andro	Etio	Andro	Etio	Andro
N	6	6	19	19	20	20
Min	-24.9	-23.3	-25.0	-24.8	-25.2	-25.5
Max	-22.7	-20.9	-22.1	-21.1	-21.9	-20.4
Mean	-23.7	-22.5	-23.3	-22.1	-23.7	-23.1
SD	0.8	1.0	0.8	0.9	1.0	1.2

We measured the  $\delta^{13}\text{C}$  values of the androstanediols for 11 of the 500 samples because one or both of the  $\delta^{13}\text{C}$  values of androsterone or etiocholanolone were more than 2 SD below the mean of the control population. The data are summarized in Table 3.

Table 3.

Gender	T/E Group	$\delta^{13}\text{C}$ (‰)					Difference from 5 $\beta$ -P		Diols would be reported as
		Etio	Andro	5 $\beta$ -Diol	5 $\alpha$ -Diol	5 $\beta$ -P	5 $\beta$ -Diol	5 $\alpha$ -Diol	
F	High	-25.2	-25.5	-26.5	-25.4	-26.2	0.2	-0.8	
F	High	-25.0	-25.0	-26.2	-27.1	-25.8	0.4	1.3	
M	High	-25.1	-25.8	-24.8	-26.8	-24.3	0.6	2.6	
M	~1	-25.9	-23.1	-24.8	-24.3	-23.8	0.9	0.5	
F	High	-24.0	-24.2	-25.6	-25.5	-24.5	1.1	0.9	
M	High	-25.3	-24.1	-26.0	-25.8	-24.7	1.3	1.1	
M	High	-25.8	-22.5	-23.5	-22.4	-21.6	1.8	0.7	
F	~1	-25.0	-24.8	-29.0	NA	-25.0	4.0	NA	Indeterminate
M	High	-28.6	-29.1	-29.7	NA	-24.0	5.7	NA	Indeterminate
M	High	-26.3	-24.6	-29.8	-28.2	-23.4	6.4	4.8	Adverse finding
M	High	-24.5	-24.6	-30.4	-30.9	-22.8	7.6	8.1	Adverse finding

Four of the 11 samples had  $\delta^{13}\text{C}$  values of one or both of the androstane diols and differences from the 5 $\beta$ -pregnanediol more than 3 SD beyond the mean of our control population(1). Due to low urinary concentrations, the  $\delta^{13}\text{C}$  value of the 5 $\alpha$ -androstane diol could not be measured in 2 of these 4 samples and therefore according to our criteria would be reported as indeterminate while the remaining 2 samples could be reported as adverse findings arising from administration of testosterone or a related compound. The data of the female with a T/E~1 are consistent with a user of any T & E product designed to either keep the T/E of a T user below 6 or replenish urinary androgens when the endogenous ones are suppressed by exogenous androgen use.

**Main Finding 3** The  $\delta^{13}\text{C}$  values for epitestosterone have been measured in 100 athletes, 51 of which screened with epitestosterone concentrations in excess of 150 ng/mL. The data are summarized in Table 4.

Table 4.

	Epitestosterone ng/mL		$\delta^{13}\text{C}$	
	Others	High	Others	High
N	49	51	49	51
Min	17	169	-23.2	-29.7
Max	56	374	-18.5	-18.8
Mean	34	223	-20.6	-21.3
SD	7	48	1.1	1.9

Three of the 100 athletes had  $\delta^{13}\text{C}$  values for epitestosterone more than 3 standard deviations below our published healthy control group of 43 males(2). In order to assess the possibility that epitestosterone had been administered to mask testosterone administration, the  $\delta^{13}\text{C}$  values of other endogenous steroids were measured. The  $\delta^{13}\text{C}$  values of the androstane diols and the differences between them and the  $\delta^{13}\text{C}$  values of the 5 $\beta$ -pregnanediol were found to be within the normal range(1), which fails to indicate testosterone use. Yet all three samples are unusual in that the differences between the  $\delta^{13}\text{C}$  values of epitestosterone and the 5 $\beta$ -pregnanediol are in excess of 4 ‰. We do not have reference data for the difference in  $\delta^{13}\text{C}$  value between

epitestosterone and the 5 $\beta$ -pregnanediol, but the data from these 3 may indicate epitestosterone administration(2;3). Although epitestosterone concentrations have been shown to increase after administration of androstenedione(4), neither the  $\delta^{13}\text{C}$  values of the androstenediols nor the differences between them and the  $\delta^{13}\text{C}$  values of the 5 $\beta$ -pregnanediol(?) provide any evidence that androstenedione was used.

### Summary of Main Findings I

Control populations have been established for the  $\delta^{13}\text{C}$  values androsterone and etiocholanolone which will aid in the interpretation of screening data from athletes' urine samples. This is especially important for cases where the measurement of the  $\delta^{13}\text{C}$  values of the androstenediols is not possible due to low concentration. The difference between the mean  $\delta^{13}\text{C}$  value of androsterone and that of etiocholanolone is significant and these values are correlated. Outliers with respect to this difference would indicate administration of steroids such as 5 $\alpha$ -dihydrotestosterone. This measurement could serve as an alternative to other confirmation procedures(5).

The results of our study which classified athletes' samples into 3 categories based on screen T/E indicate that a reduction of the cutoff for a T/E positive from 6 to 4 may increase detection of doping. Using a screen of the  $\delta^{13}\text{C}$  values of androsterone and etiocholanolone we found four out of 500 samples which merited further study. Two of those four samples could be reported as adverse findings using our accepted criteria for the  $\delta^{13}\text{C}$  values of 2 androstenediols and 1 pregnanediol. Both of these samples had a screen T/E between 4 and 6 and according to WADA guidelines effective August 13, 2004 would have been submitted for IRMS analysis(6).

The  $\delta^{13}\text{C}$  values of epitestosterone in 3 of 49 samples which had screened with epitestosterone concentrations in excess of 180 ng/mL were found to differ from the  $\delta^{13}\text{C}$  value of the 5 $\beta$ -pregnanediol by more than 4 ‰. Although this difference is highly suspect and could represent administration of epitestosterone as a masking agent(7), a measurement of the  $\delta^{13}\text{C}$  values of the androstenediols did not indicate testosterone administration. Yet the simultaneous administration of testosterone and epitestosterone should not be ruled out as the relationship between the elimination half-lives of testosterone and epitestosterone is not known.

2) *If the projected timeline has not been achieved, do changes need to be made to the aim or objective?* No

3) *What are your plans for the project next year?* This is the final report.

4) *If you are working in collaboration with others, or depend on other organizations or institutions to meet the objectives, how are those relationships working?* NA

**Specific Aim II:** To compare urinary metabolites, T/E ratios, and plasma testosterone after administering oral testosterone to males with high T/E ratios and those with low T/E ratios.

***Hypothesis:*** There will be large and significant differences in the outcome measures in the two groups of subjects. Those in the high mode (HM-T/E) group will have typical rises in plasma

testosterone, rapid elevations in urine T/E, high excretion rates of testosterone and its metabolites, and changes in  $\delta^{13}\text{C}$  values consistent with full incorporation of the exogenous testosterone into the metabolic pathways. Conversely the subjects in the low mode (LM-T/E) group will have minimal rises in plasma testosterone, no change in urine T/E, low excretion rates of urine testosterone and metabolites, and uncertain changes in  $\delta^{13}\text{C}$  values of urinary steroids.

### *1) What progress has been made?*

Our initial plan was to use a cyclodextrin testosterone preparation that could be given orally. This type of preparation has still not been licensed in the United States and research material could not be obtained. As stated in our initial proposal we moved to a transdermal administration.

Potential subjects were screened for baseline T/E with a random urine collection. Those subjects who had TE < 0.3 or > 1.0 were invited to participate in the study. Nine subjects began the study, 5 HM and 4 LM; however, one of the HM subjects (HM 4) had the patches fall off shortly after the study began and was excluded from further analysis. The subjects are described in Table 5.

Table 5.

	T/E	Age	Ht (in)	Wt (lbs)
LM1	0.1	30	69	175
LM2	0.2	20	66	130
LM3	0.2	20	66	155
LM4	0.2	22	69	140
HM1	1.1	25	70	165
HM2	1.6	26	66	150
HM3	2.7	25	74	230
HM4	4.9	26	66	163
HM5	6.3	25	76	180

The study was approved by the UCLA IRB (#02-11-083-02A) and all subjects gave written informed consent. A medical screening questionnaire was completed: subjects were free from any medical problems and had no illnesses affecting their kidneys, liver, or reproductive hormones. None of the subjects were taking any prescription medications or nutritional supplements.

On Day 1 the subjects reported at approximately 8 am, were given urine collection vessels, and were instructed to do 2 four-hour urine collections, i.e., from 8-12 noon and 12 noon to 4 pm. Prior to beginning the first collection, subjects went to the restroom and emptied their bladders. Subjects reported back at approximately 4 pm with their collection containers. On Day 2, the subjects again reported at approximately 8 am. A baseline blood sample was taken and subjects were again asked to empty their bladders completely. Serum was aliquotted and sent to the UCLA Clinical Lab for a comprehensive metabolic panel. The UCLA Olympic Analytical Laboratory analyzed a serum aliquot by LC-MS for testosterone. A physician applied 4 patches



each containing 5 mg testosterone (Androderm® by Watson Pharma, Corona, CA, lot #91340804) as recommended by the manufacturer to a glabrous area of the back. If the patches fell off, the subjects were instructed to contact the researcher and new patches were applied. Subjects were instructed to perform 2 four-hour urine collections. Subjects returned at approximately 4 pm and had a second venipuncture performed and one serum separator tube of blood collected. They were given urine collection vessels for a four-hour collection (4 pm to 8 pm) and then for an overnight collection (8 pm to 8 am). On Day 3 the subjects reported at approximately 8 am and had a third venipuncture and one serum separator tube of blood collected (about 5 mL). The testosterone patches were removed, the skin wiped down with alcohol and the subjects were given urine collection vessels for the two final four-hour urine collections. Subjects returned at approximately 4 pm to return the urine containers and complete the study.

Urinary concentrations of androsterone, etiocholanolone, testosterone, epitestosterone, 5 $\beta$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (5 $\alpha$ -diol), 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (5 $\beta$ -diol), epitestosterone precursor, epitestosterone M1, and epitestosterone M2 were calculated by GCMS by comparison to a standard curve prepared by spiking a steroid-free urine matrix. Plasma testosterone glucuronide concentrations were determined by LC/MS/MS(8).

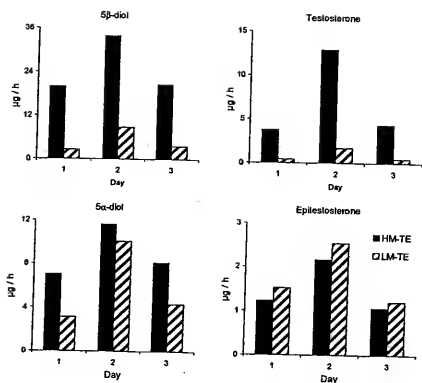
The  $\delta^{13}\text{C}$  values of urinary androsterone, etiocholanolone, 5 $\beta$ -androstane-3 $\alpha$ ,17 $\beta$ -diol, 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol 5, and 5 $\beta$ -pregnane-3 $\alpha$ ,20 $\alpha$ -diol (5 $\beta$ -P) were determined according to our published methods(9;10). Due to low urinary concentrations, the  $\delta^{13}\text{C}$  values of 5 $\beta$ -diol, 5 $\alpha$ -diol, and 5 $\beta$ -P could not be measured in two samples.

Statistical comparisons between day and groups were conducted via mixed-effect ANOVA using MIXED(11) procedure on SAS version 8.02 (SAS Institute, Cary, NC).

**Main Finding 1** Testosterone extracted from one patch of the same lot used for the study was found to have a  $\delta^{13}\text{C}$  value of -28.8 %.

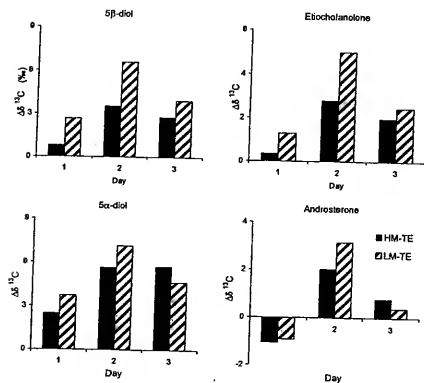
**Main Finding 2** The mean day 2 testosterone excretion rate was significantly higher than on day 1 for the HM group ( $p=0.0002$ ) but not the LM group. Mean testosterone excretion rates differed between HM and LM groups on day 2 ( $p=0.0004$ ). Epitestosterone excretion rate differed between day 1 and day 2 for all subjects combined ( $p=0.0204$ ) but not within groups. Mean day 2 5 $\alpha$ -diol excretion rates were different from day 1 for both the HM ( $p=0.0155$ ) and LM ( $p=0.0102$ ) groups. Mean day 2 5 $\beta$ -diol excretion rates were different from day 1 for the HM ( $p=0.0018$ ) but not the LM group. Mean 5 $\beta$ -diol excretion rates differed between HM and LM groups on days 1 ( $p=0.0042$ ) and 2 ( $p=0.0004$ ). Mean excretion rates by day and group are presented below (Figure 2).

Figure 2.



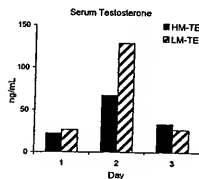
**Main Finding 3** Differences in  $\delta^{13}\text{C}$  values ( $\Delta\delta^{13}\text{C}$ ) for androsterone, etiocholanolone,  $5\alpha$ -diol and  $5\beta$ -diol relative to  $5\beta$ -P are presented below (Figure 3). HM and LM groups are different only on day 2 and then only for  $5\beta$ -diol. Within the LM group day 2  $\Delta\delta^{13}\text{C}$  was increased on day 2 and returned near baseline levels for all 4 compounds on day 3. The pattern is somewhat different within the HM group with no significant change seen for etiocholanolone, increased  $\Delta\delta^{13}\text{C}$  on day 2 for androsterone and increased  $\Delta\delta^{13}\text{C}$  on days 2 and 3 for  $5\alpha$ -diol and  $5\beta$ -diol.

Figure 3.



**Main Finding 4** Mean serum testosterone concentration increased on day 2 and returned to baseline levels on day 3 for both the LM ( $p=0.0003$ ) and HM ( $p=0.0036$ ). While there was no difference in baseline serum testosterone concentrations, the mean concentration for the LM group (129 ng/mL) was significantly higher ( $p=0.0386$ ) than that of the HM group at 129 and 67 ng/mL, respectively (Figure 4).

Figure 4.



### Summary of Main Findings II:

The measured  $\delta^{13}\text{C}$  value of  $-28.8\text{‰}$  for testosterone extracted from one 5 mg patch marketed under the name Androderm® by Watson Pharma is similar to that of commercially available reference standards.

Upon transdermal testosterone administration, HM subjects exhibit increased testosterone excretion rates while LM subjects do not although serum testosterone concentrations are elevated for both groups. While  $5\alpha$ -diol excretion rates are increased for both HM and LM groups, only the HM group exhibited significantly increased  $5\beta$ -diol excretion rates.

With respect to  $\Delta\delta^{13}\text{C}$  for  $5\alpha$ -diol and  $5\beta$ -diol, levels returned to baseline 24 hours after administration for the LM group but remained significantly higher than baseline for the HM group.

2) *If the projected timeline has not been achieved, do changes need to be made to the aim or objective?*

3) *What are your plans for the project next year?* This is the final report.

4) *If you are working in collaboration with others, or depend on other organizations or institutions to meet the objectives, how are those relationships working?* NA

**Specific Aim V:** To conduct a cross-sectional study of the influence of diet on the  $\delta^{13}\text{C}$  values of 5 urinary steroids and to conduct a longitudinal study of the influence of altering the diet from: normal→ high soy→ normal on the  $\delta^{13}\text{C}$  values of urinary steroids.

**Hypothesis:** The  $\delta^{13}\text{C}$  values of urinary steroids in subjects whose diet consists mainly of vegetable products will be lower than the  $\delta^{13}\text{C}$  values of urinary steroids of a comparable control

group that ingests a normal diet. In the longitudinal study the  $\delta^{13}\text{C}$  values of urinary steroids will be in the normal range during the baseline period, they will decrease while on the soy diet, and return to normal during the recovery period. The changes in  $\delta^{13}\text{C}$  values will not be sufficient to result in "positive" IRMS reports.

### 1) What progress has been made?

The cross-sectional study has been completed. A total of 20 control subjects and 24 vegan subjects completed the urine collection. One control subject, who submitted a dilute sample that could not be analyzed, was excluded. Only 15 of the remaining control subjects completed diet histories. Following their analysis for soy content, one control subject was excluded because he obtained too many calories (at least 10%) from soy. Of the 24 vegan subjects who completed the urine collection, one subject did not complete the 7-day diet history and one subject's dietary analysis revealed inadequate total calories (35% of predicted) indicating an incomplete recording. Both were excluded from the study. This yielded a total of 14 control subjects and 22 vegan subjects for comparison. The data are summarized in tables 6 and 7. Measured  $\delta^{13}\text{C}$  values of urinary androsterone and etiocholanolone for the vegan group were significantly more negative ( $p < 0.001$ ) than controls. Urinary concentrations did not significantly differ between groups ( $p > 0.1$ ).

Table 6.

	CONTROL N=18				VEGAN N=23			
	AGE	GENISTEIN	DAIDZEIN	TOTAL	AGE	GENISTEIN	DAIDZEIN	TOTAL
MIN	22	0	38	38	15	207	184	391
MAX	38	3394	2346	5740	49	40015	24912	64927
MEDIAN		213	205	485		1966	1224	3227
MEAN	29	688	436	1124	28	6103	3376	9479

Table 7.

	CONTROL N=18				VEGAN N=23			
	ETIO		ANDRO		ETIO		ANDRO	
	ng/mL	$\delta^{13}\text{C}$	ng/mL	$\delta^{13}\text{C}$	ng/mL	$\delta^{13}\text{C}$	ng/mL	$\delta^{13}\text{C}$
MIN	735	-25.3	933	-23.8	141	-27.2	285	-25.4
MAX	3057	-22.4	5832	-21.0	4012	-22.5	9268	-22.1
MEAN	2175	-23.7	2285	-22.3	1849	-24.9	2695	-23.6
CV%	34	3	58	3.5	59	4	82	4

A Longitudinal pilot study to assess the rate of change of  $\delta^{13}\text{C}$  values of urinary steroids upon a change in diet has been conducted. One healthy male subject, 37 years of age collected 24 hour urines for 24 days. On days 2 through 14, the subject consumed a corn-free diet while on all other he ate as usual. On days 17-19 and 23, no urine was collected. Urinary concentrations of androsterone, etiocholanolone, testosterone, epitestosterone,  $5\beta$ -diol,  $5\alpha$ -diol, and DHEA were calculated by GCMS by comparison to a standard curve prepared by spiking a steroid-free urine matrix. The  $\delta^{13}\text{C}$  values of urinary androsterone, etiocholanolone,  $5\beta$ -diol,  $5\alpha$ -diol, and  $5\beta$ -P were determined according to our published methods(9;10).

**Main Finding 1** Both the control and vegan groups were similar to the laboratory's reference population in terms of their average T/E ratio. The T/E ratio ranged from 0.04 to 3.21 in the vegan group and from 0.08 to 3.15 in the control group. There was a statistical difference between the groups with respect to T/E ( $p=0.02$ ).

**Main Finding 2** The mean  $\delta^{13}\text{C}$  values for androsterone and etiocholanolone in the vegan group were -23.6 and -24.9 ‰, respectively whereas in the control group they were -22.5 and -23.9‰. The  $\delta^{13}\text{C}$  values were significantly different between control and vegan groups for both androsterone ( $p=0.0007$ ) and etiocholanolone ( $p=0.005$ ). Three subjects in the vegan group had  $\delta^{13}\text{C}$  values for one or both of androsterone and etiocholanolone removed in the negative direction by more than three standard deviations from the laboratory reference means (-24.8 for androsterone and -26.1 etiocholanolone). These samples underwent IRMS analysis for  $5\beta$ -diol,  $5\alpha$ -diol, and  $5\beta$ -P. No differences (Table 8) between the androstenediols and the pregnanediol exceeded our 3 S.D. cutoff. Differences between both androsterone and etiocholanolone and the pregnanediol did not exceed 3 ‰ and would therefore not require a report of "consistent with the administration of a steroid" (6).

Table 8.

			$\delta^{13}\text{C}$ (‰)					Difference from 5 $\beta$ -P			
Age	Sex	T/E	Etio	Andro	5 $\beta$ -Diol	5 $\alpha$ -Diol	5 $\beta$ -P	5 $\beta$ -Diol	5 $\alpha$ -Diol	Etio	Andro
47	F	0.21	-27.2	-25.4	-24.4	-25.6	-25.5	-1.2	0.0	1.7	-0.1
44	M	0.78	-26.8	-25.1	-26.3	-27.0	-25.7	0.6	1.3	1.1	-0.6
49	M	1.32	-26.0	-25.4	-26.0	-26.0	-25.1	1.0	1.0	0.9	0.3

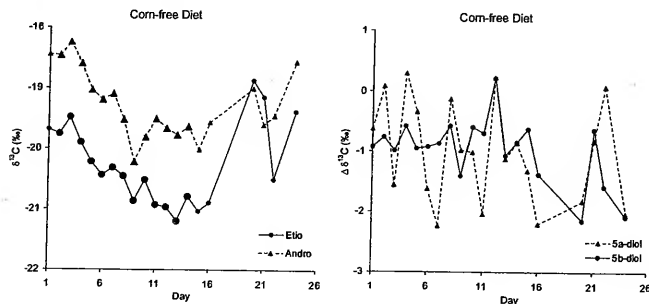
**Main Finding 3** No correlations were found between  $\delta^{13}\text{C}$  values and collected dietary parameters, specifically carbohydrate, fat and protein in diet expressed both in terms of grams and percent of total intake, total kcal, kcal from soy, grams of soy protein, percent of soy carbohydrate, protein and fat, and percent of total protein derived from soy. No correlations were found between  $\delta^{13}\text{C}$  values and any of fat, saturated fat, cholesterol, or dietary fiber (in either the total or soy portion of the diet).

**Main Finding 4** The isoflavone analysis was carried out on 14 control and 22 vegan subjects. The results are summarized in Table 6. The vegan group's median isoflavone excretion rate at 23  $\mu\text{mol/day}$  was significantly higher ( $p=0.0002$ ) than the 2.7  $\mu\text{mol/day}$  in the control group. The isoflavone values were also compared with the dietary analyses and the best correlation was between total isoflavones excreted per day and the amount of fat derived from the soy portion of the vegetarian subject's diet ( $R=0.56$ ). There was otherwise no correlation between the amount of isoflavones excreted per day and the 7-day dietary recall. The amount of isoflavones excreted did not correlate with IRMS values for either androsterone or etiocholanolone ( $R=-0.09$  and  $-0.04$ , respectively).

**Main Finding 5** Although the plot of  $\delta^{13}\text{C}$  values appears to have a downward trend during the shift to a corn-free diet (Figure 4), the mean  $\delta^{13}\text{C}$  values for both androsterone and etiocholanolone were not significantly different when comparing days on the control and corn-

free diets ( $p > 0.05$ ). There is no apparent effect of diet on the differences between the androstanediols and the pregnanediol (Figure 4).

Figure 4.



#### Summary of Main Findings V:

In the cross-sectional study a statistical difference was found between the control and vegan populations with respect to T/E ratio. Mean  $\delta^{13}\text{C}$  values for androsterone and etiocholanolone were also significantly between groups.

Although three subjects in the vegan group had  $\delta^{13}\text{C}$  values for one or both of androsterone and etiocholanolone removed in the negative direction by more than three standard deviations from the laboratory reference means,  $\Delta\delta^{13}\text{C}$  for 5 $\alpha$ -diol and 5 $\beta$ -diol relative to 5 $\beta$ -P did not exceed our 3 S.D. cutoff(1).

No correlations were found between  $\delta^{13}\text{C}$  values and any collected dietary parameters. Neither did  $\delta^{13}\text{C}$  values correlate with measured urinary isoflavone concentrations.

Our longitudinal pilot study showed no significant differences between  $\delta^{13}\text{C}$  values of androsterone, etiocholanolone, 5 $\alpha$ -diol or 5 $\beta$ -diol.

2) *If the projected timeline has not been achieved, do changes need to be made to the aim or objective?*

3) *What are your plans for the project next year?* This is the final report.

4) *If you are working in collaboration with others, or depend on other organizations or institutions to meet the objectives, how are those relationships working?* NA

Results: A manuscript describing the results of our cross-sectional study is being prepared.

2) *If the projected timeline has not been achieved, do changes need to be made to the aim or objective?* No

3) *What are your plans for the project next year?* This is the final report.

4) *If you are working in collaboration with others, or depend on other organizations or institutions to meet the objectives, how are those relationships working?* NA

**FOR THE OVERALL RESEARCH PROJECT, ANSWER THE FOLLOWING:**

1) *What have been the project's key dissemination activities during the past year?* None.

2) *Have there been changes to the project's other sources of support?* No.

**QUESTIONS THAT PERTAIN TO THE FINAL REPORT**

1) **Are there actions that need to be taken to facilitate incorporation of the results of your research into routine drug testing?** There are no specific issues but we should chat about some of the implications, particularly with respect to TE.

2) **Do you have recommendations for additional dissemination of the results of your research that USADA should pursue?** I can see that Dr. Bowers is involved in international aspects of the method in general - reference standards, etc. This is very useful. I also note the formation of a new science advisory committee which is also very much needed.

3) **How do you assess USADA's role.** In addition to providing the funding USADA has been a great help in many ways. They have made certain sample available to us. Many of my particular issues regarding research funding are under discussion at USADA. This is particularly important to me and I trust the effort will continue.

An issue that deserves serious reflection pertains to privacy and confidentiality. I am sure that USADA does the best it can under the circumstances, but in light of Balco I wonder if it is time to try a different approach. The dopers are a determined lot, they have no scruples, and they do not obey any rules. They use a variety of known and unknown techniques to obtain our methods and use them for nefarious purposes. Efforts to limit their success are needed.

**Reference List: Laboratory Publications and Work-Products attributed in part to the USADA IRMS Grant**

Reference List

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2. Aguilera R, Hatton CK, Catlin DH. Detection of epitestosterone doping by isotope ratio mass spectrometry. Clin Chem 2002 ;48 (4):629 -36 2002;48:629-36.

3. Starka L. Epitestosterone. *J Steroid Biochem Mol Biol* 2003 Oct ;87 (1):27 -34 2003;87:27-34.
4. Catlin DH, Leder BZ, Ahrens BD, Hatton CK, Finkelstein JS. Effects of androstenedione administration on epitestosterone metabolism in men. *Steroids* 2002 Jun ;67 (7 ):559 -64 2002;67:559-64.
5. Kicman AT, Coutts SB, Walker CJ, Cowan DA. Proposed confirmatory procedure for detecting 5 alpha-dihydrotestosterone doping in male athletes. *Clin Chem* 1995;41:1617-27.
6. WADA Laboratory Committee. Reporting and Evaluation Guidance for Testosterone, Epitestosterone, T/E ratio, and Other Endogenous Steroids. TD2004EAAS. 2005.  
Ref Type: Internet Communication
7. Catlin DH, Hatton CK, Starcevic SH. Issues in detecting abuse of xenobiotic anabolic steroids and testosterone by analysis of athletes' urine. *Clin Chem* 1997;43:1280-8.
8. Starcevic B, DiStefano E, Wang C, Catlin DH. Liquid chromatography-tandem mass spectrometry assay for human serum testosterone and trideuterated testosterone. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003 Jul 25 ;792 (2):197 -204 2003;792:197-204.
9. Aguilera R, Catlin DH, Becchi M, Phillips A, Wang C, Swerdloff RS et al. Screening urine for exogenous testosterone by isotope ratio mass spectrometric analysis of one pregnanediol and two androstane diols. *J Chromatogr B Biomed Sci Appl* 1999;727:95-105.
10. Aguilera R, Chapman TE, Catlin DH. A rapid screening assay for measuring urinary androsterone and etiocholanolone delta(13)C ( per thousand) values by gas chromatography/combustion/isotope ratio mass spectrometry. *Rapid Commun Mass Spectrom* 2000 ;14(23):2294 -9 2000;14:2294-9.
11. SAS System for Linear Models, Third ed. Cary, NC: SAS Institute Inc., 1991.





# UNITED STATES ANTI-DOPING AGENCY

## GRANT APPLICATION

**1. Title of Project**

Standardization and Methodology for Steroid Isotopic Analysis

**2. Principal Investigator/Program Director****2a. Name (last, first, middle)**

Brenna, James Thomas

**2b. Degrees(s)**

BS MS PhD

**2c. Social Security Number**

041-50-7417

**2d. Position Title**

Professor and Director of Undergraduate Studies

**2e. Mailing Address (street, city, state, zip code)**

Cornell University

Savage Hall

Ithaca, NY 14853

**2f. Department, Service, Laboratory, or Equivalent**

Division of Nutritional Sciences

**2g. Major Subdivision**



NYS Colleges of Human Ecology &amp; Ag &amp; Life Sciences

**2h. Telephone and Fax (area code, number and extension)**

(607)255-9182 ; Fax 255-1033

**2i. Email**

&lt;jtb4@cornell.edu&gt;

<b>3. Human Subjects</b> <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<b>3a. If "Yes" IRB approval date:</b>	<b>Full IRB or Expedited Review</b> <input type="checkbox"/> Yes <input type="checkbox"/> No	<b>3b. Assurance of compliance number:</b>	<b>4. Vertebrate Animals</b> <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<b>4a. If "Yes" IACUC approval date:</b>	<b>4b. Animal welfare assurance number:</b>
<b>5. Costs requested for Initial Budget Period: (will be automatically entered from Budget Page)</b> <b>5a. Direct Costs (\$):</b> 554,483 <b>5b. Total Costs (\$):</b> 693,104				<b>6. Costs requested for Proposed Period of Support: (will be automatically entered from Budget Page)</b> <b>6a. Direct Costs (\$):</b> 1,075,790 <b>6b. Total Costs (\$):</b> 1,344,737		
<b>7. Applicant Organization:</b> <b>Name</b> Cornell University <b>Address</b> Office of Sponsored Programs 120 Day Hall Ithaca, NY 14853-2801				<b>8. Dates of Proposed Period of Support (month/day/year - MM/DD/YYYY)</b> <b>From</b> 1 Feb 2006 <b>Through</b> 31 Jan 2009		
<b>10. Official Signing for Applicant Organization:</b> <b>Name</b> Brenda Truesdail <b>Title</b> Sr. Grant & Contract Officer <b>Address</b> Cornell University Office of Sponsored Programs 120 Day Hall Ithaca, NY 14853-2801 <b>Telephone</b> 607-255-7124 <b>Fax</b> 607-255-5058 <b>Email</b> bmt2@cornell.edu				<b>9. Type of Organization</b> <b>Public:</b> <input type="checkbox"/> Federal <input type="checkbox"/> State <input type="checkbox"/> Local <b>Private:</b> <input checked="" type="checkbox"/> Private Nonprofit <input type="checkbox"/> Private Forprofit <b>11. Administrative Official to be Notified if Award is Made (if different than "Official Signing":</b> <b>Name</b> Brenda Truesdail <b>Title</b> Sr. Grant & Contract Officer <b>Address</b> Cornell University Office of Sponsored Programs 120 Day Hall Ithaca, NY 14853-2801 <b>Telephone</b> 607-255-7124 <b>Fax</b> 607-255-5058 <b>Email</b> bmt2@cornell.edu		
<b>12. Principal Investigator/Program Director Assurance:</b> I certify that all expenditures made with grant funds awarded by USADA are for appropriate grant purposes in furtherance of USADA's scientific and educational purposes and in accordance with the provisions of the application, grant terms and conditions and all other award documents.				<b>Signature of PI/PPD Named in 2a.</b> 		<b>Date</b> 3/04/05
<b>13. Applicant Organization Certification and Acceptance:</b> I certify that all expenditures made with grant funds awarded by USADA are for appropriate grant purposes in furtherance of USADA's scientific and educational purposes and in accordance with the provisions of the application, grant terms and conditions and all other award documents.				<b>Signature of Official Named in 10.</b> 		<b>Date</b> 11-1-05

Type the name of the principal investigator/program director at the top of each printed page and each continuation page.

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**a. Specific Aims**

The high precision isotopic analysis of endogenous steroids is now recognized as a unique and powerful tool for detection of doping. The analysis is based on gas chromatography combustion isotope ratio mass spectrometry (GCC-IRMS) of several different steroids. As currently practiced, GCC-IRMS continues to be a specialty technique that is considered difficult to standardize for routine use by high throughput labs.

The purpose of this proposal is a focus on the analytical chemistry of high precision isotope measurements for steroid analysis. Protocols, standards, and software will be developed for comprehensive harmonization of these analyses that can be implemented in testing laboratories worldwide, and concurrently to research a cutting edge strategy for improving the sensitivity and specificity of analysis. The conclusions of the 2003 USADA Research Symposium that dealt with these issues extensively will guide the project, and the researchers expect to be in close contact with USADA during the course of the project.

To these ends, the specific aims are:

- 1) To create isotopic standards of several types for the commonly analyzed steroids.
  - o Round robin standards
  - o Proficiency standards
  - o Working standards
- 2) Instrument-independent software to enable cross-platform comparison of isotopic data.
- 3) A basic research component into the compound-specific analysis of steroids to improve accuracy and precision of analysis and reduce the importance of inferring compounds. This will consist of the first interfacing of the novel technique of GCxGC (2 dimensional GC) as a separation system for IRMS, to eliminate interferences in complex matrices.

At the conclusion of this 3 year program, we will have a) developed and made available to the doping testing community a series of standards for calibration and proficiency testing, b) conducted at least one round robin test of worldwide proficiency in isotopic steroids testing, and c) investigated whether a cutting edge strategy for isotopic analysis can be applied to improve steroid testing on a routine basis.

**b. Background and Significance****i. Significance:**

Recommendations for refinement of high precision isotopic testing for doping.

The Second Annual USADA Symposium on Anti-Doping Science in 2003 was titled "Application of Gas Chromatography - Combustion - Isotope Ratio Mass Spectrometry to Doping Control". It was attended by representatives of Olympic testing laboratories worldwide, officials of USADA and the US

Olympic Committee, and experts in GCC-IRMS. The conclusions of that symposium are reproduced in Box 1, from the USADA webpage [2]. Of the six main recommendations, four (1, 2, 4, 5) directly involve analytical standards and related developments, and the others would benefit from such an effort. It is the purpose of this proposal to be directly responsive to the recommendations involving analytical developments (underlines added) as deliverables from this grant, and to work with USADA through Dr Bowers and others to achieve as many of the goals as can be addressed with the resources that become available. We regard these as generally low risk tasks that nevertheless require considerable experience in IRMS analyses for success. In addition, a research component of greater risk and potentially high reward is added to the proposal, consistent with the goals of increasing the sensitivity and specificity of the analyses in goal 6.

*Box 1: 2003 USADA Research Symposium. Recommendations and Conclusions*

- 1) A small expert panel should be convened to consider what specific assay improvements are needed, guidelines should be used, and make recommendations to WADA. These recommendations may include guidelines on assay characteristics and performance criteria that will unify the method(s) that are used in doping control.
  - a) Build on the foundation provided by the IOC Working Group
  - b) Develop a Proficiency Testing Round Robin to improve harmonization of reported values
- 2) USADA should fund a 50-subject reference range study in the eleven labs currently reporting GC-C-IRMS data
  - a) Reference material and/or internal standard must be identified and produced
  - b) Target substances must be identified and run in all samples
  - c) Specify internal standard and reporting requirements
- 3) There are ways to beat the GC-C-IRMS test, such as administration of hCG, aromatase inhibitors, or clomiphene.
  - a) Doping may have occurred, even in the presence of a negative GC-C-IRMS test;
  - b) A positive GC-C-IRMS does definitively support the administration of pharmaceutical T or T precursors
  - c) An expert panel needs to provide recommendations on the combination of longitudinal testing and GC-C-IRMS to provide cost-effective testing strategies
  - d) A GC-C-IRMS may be required in every case of an elevated T/E ratio
- 4) The reporting of differences of  $\delta^{13}\text{C}$  values (using an endogenous reference compound) as opposed to the ratio of  $\delta^{13}\text{C}$  values is strongly recommended. There may be additional benefit in considering the absolute  $\delta^{13}\text{C}$  values compared to the reference ranges of individual target analytes.
- 5) A Reference Material and/or Internal Standard material should be developed to assist the laboratories in achieving more uniform results.
- 6) There is a need for additional research funding in this area. Topics include the sensitivity and specificity of screening using A/E versus the Adiolis.

In the balance of this Background section, we outline various technical aspects that involve directly or indirectly the measurement of natural isotope ratios for reference.

## ii. High precision isotope ratio mass spectrometry (IRMS) and isotopic calibration

High-precision gas isotope ratio mass spectrometry (IRMS) is the classical technique for determination of isotope fractionation due to natural processes for C, H, N, O, and S (1,2). It is the difference between isotope ratios of unknowns and that of a working standard which can be measured with great reproducibility on a day-to-day basis, and so reliable working standards, calibrated against international standards, are essential.

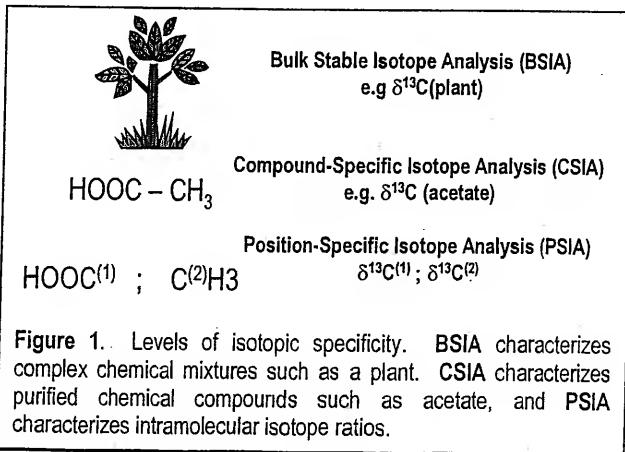
High precision isotope measurements are considered at three levels of specificity. Bulk Stable Isotope Analysis (BSIA) involved combustion or other chemical reactions that transform a chemically complex sample into  $\text{CO}_2$ ,  $\text{N}_2$  or similar small molecule for isotopic analysis. Compound-Specific Isotope Analysis (CSIA) refers specifically to isotopic analysis of an isolated chemical compound, typically by gas chromatography (GC). At the highest level of detail, position-specific isotope analysis (PSIA) measures intramolecular isotope ratios, which in principle should be most sensitive to alterations by adulteration or doping. A purely instrumental method based on IRMS is to combine online pyrolytic fragmentation with separations for PSIA analyses [3].

At the BSIA (bulk) level and at the CSIA level, the isotopic calibrant gas is usually  $\text{CO}_2$  admitted to the IRMS from a separate volume. CSIA data can also be calibrated by use of a calibrated compound within the chromatogram [4]. At the PSIA level, pyrolysis-induced fractionation requires that the calibrant is reference compound of the same structure as the analyte [5], though recent progress in our laboratory suggests that calibrant gas might be used as well (Wolyniak and Brenna, unpublished).

High precision isotope analysis of steroids is done at the CSIA level, and thus  $\text{CO}_2$  is most often used. However, the availability of calibrated steroids would open an alternative method, and in addition would produce standards for various types of analyses. This alternative is considered preferable for some analyses since standard would be subject to equivalent chemical steps as the analyte.

## iii. Isotopic Reference Materials – Creation and use

The properties for high precision isotope standards were first enunciated by A. Nier [6] and the first reference material for carbon analysis, a carbonate known as PeeDee Belemnite (PDB) with  $R_{\text{PDB}} = {}^{13}\text{C}/{}^{12}\text{C} = 0.0112372 \pm 0.0000090$  (3) which is relatively high for natural materials. Although the supply of this material has long been exhausted, carbon isotopic standards generated since PDB are calibrated against this figure and expressed using the standard



$\delta^{13}\text{C}_{\text{PDB}} = 1000 \times (R_{\text{spl}} - R_{\text{PDB}}) / R_{\text{PDB}}$ , where  $R_{\text{SPL}}$  is the carbon isotope ratio of the sample. The chemical composition of most carbon standards, for instance, oil, polyethylene foil, or graphite, is not fully characterized [7], because they are completely combusted prior to use. They therefore can serve as CSIA standards only insofar as they are used to calibrate a working standard of  $\text{CO}_2$  gas.

CSIA standards have been created, notably for natural gases and for both C and H, by combusting an aliquot of pure compounds (e.g., methane, ethane, propane), characterizing their isotope ratios, and then mixing them together.

This procedure has been rarely used for biological CSIA. A barrier to creation of standards for endogenous compounds is that there is always some endogenous analyte of necessarily unknown isotope ratio in every sample. Addition of an isotopically calibrated compound would then result in an unknown overall isotope ratio. The way around this problem is to use such a procedure with 2 or more isotopically calibrated standards to calibrate the endogenous isotope ratio by the method of standard addition. This procedure is very similar to the method of standard addition for quantitative calibration, except that the standard would be added by a very careful quantitative measurement, and then the ratio of standard to endogenous analyte used to calculate the isotope ratio of the endogenous material. In total then, the isotope ratio of the analyte within matrix, e.g. urine, can then be used as a standard. We will use a procedure employing these principles to create standards for steroid analysis.

Round Robin ("Interlaboratory Comparisons" or "Ring Tests"). A general method for worldwide calibration of standards is the Round Robin, or Ring Test method. The method has been used numerous times for calibration of isotopic standards (e.g., [8-10]). In this method, replicate samples of known, very low variability are distributed to laboratories for analysis. Laboratories are masked to the isotope ratios, analyze them according to their standard methods for unknowns, and return values to a central recorder along with a secret identifier code. The recorder removes information connecting the isotope ratio results and the specific laboratory and passes the results with codes to the central research laboratory. Results are analyzed and circulated with codes only so that specific laboratories can compare their results to worldwide results, and all can compare the summary data, including mean and distribution of results for quality control and for establishing minimal levels of proficiency. Development of a Round Robin program is responsive to Recommendation 1 of Box 1.

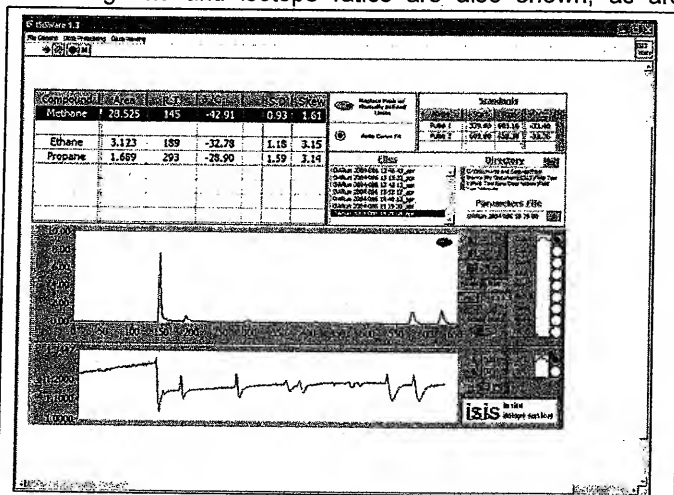
#### iv. IRMS Data processing

IRMS instruments generate an array of raw data from their faraday cup detectors that must be converted to isotope ratios. The algorithms for performing this function are highly refined but are generally not known to the user community because they are generally developed by instrument manufacturers and held as proprietary. Only very general descriptions exist in the open literature (e.g., ref. [11]). The Brenna group at Cornell has published extensively in this area (e.g., ref. [12-14]) and has developed software that is currently running routinely to process IRMS data by both the conventional summation method, as is done on all commercial IRMS instruments, and by the curve-fitting method, which we have shown to be more robust to peak overlaps [13], low signal levels [14], as well as limitations in instrument hardware [12]. We are also able to import raw data into our software from multiple instrument manufacturers and process it on a single platform (Sacks and Brenna, unpublished), and to customize software to particular applications.

This capability has been used to create software for a private firm for analysis of natural gas (unpublished) and could be adapted for any application. Figure 2 is a screen capture from this software. The software is written in the versatile laboratory programming language LabView (National Instruments) and the core code is available for adaptation for any purpose. For instance, in Fig. 2, the application is isotopic analysis of natural gas components by GCC-IRMS. The instrument manufacturer's software acquires data and outputs a simple text (ASCII) file of raw data onto the hard disk. Our software reads this data and then processes it as customized for the specific application. This strategy avoids conflicts with proprietary software code that is often kept secret by vendors, and allows our software to operate with any raw data that can be outputted to a text file. We also have implemented direct acquisition of data through vendor file codes when we are able to get the code from the manufacturer, and have another program than reads data files in native format from Thermo-Finnigan, APP (now a part of GV), and the now-defunct manufacturer PDZ Europa. The software shown in Figure 2 can process data with the conventional summation algorithm that is used in all commercial IRMS software. It can also perform curve-fitting using the Exponentially-Modified Gaussian (EMG) function, and can easily be modified to use any desired function. It has been tailored to permit users to select regions in which compounds of interest appear, and then in a batch mode find the relevant peaks and calculate isotope ratios against calibrant gas, appearing in the figure as the two large late eluting peaks. In this example, isotope ratios from 3 components (methane (-42.91), ethane (-32.78), and propane (-28.90)) are calculated using curve-fitting and are shown in the upper left box. The center box labeled "Files" shows a series of filenames that were processed automatically. Plots of chromatograms and isotope ratios are also shown, as are parameters associated with the standards in the upper right box. Software similar to this could be developed for any application, including steroid testing and implementation on multiple platforms would be straightforward.

#### v. Testosterone testing

Isotopic testing of testosterone in urine for doping has been described in many papers published over more than a decade in humans and for cattle [15-34]. The technique and its variants were discussed extensively at the USADA symposium referenced above. The core idea is that the  $\delta^{13}\text{C}$  of synthetic testosterone is different from that of naturally produced testosterone, due to differences in source carbon [35]. By this principle, the use of exogenous (doped) testosterone can be detected by measuring the



**Figure 2.** Screen capture of software developed by members of the Brenna group at Cornell for a private IRMS service company (ISIS, Inc.). Raw data is imported from an ASCII text file and isotope ratios can be calculated for selected components using the conventional summation algorithm or by curve-fitting with the exponentially modified Gaussian (EMG) function. The core code is fully available to be adapted for other applications.

isotope ratio of an endogenous reference steroid in the precursor pathway to testosterone, along with either testosterone itself or a metabolite for convenience. Several schemes have been proposed over the years. Two methods that are in present use require the analysis of steroid pairs *androsterone/etiocholanolone* (ando/etio) or  $\alpha/\beta$ -*androsteroids* ( $\alpha/\beta$ ) in urine. It is conceivable that other pairs might emerge as of interest. We will however focus the proposal discussion on these four steroids, and methods for others will apply with minor modifications.

#### vi. Analytical Chemical Research: GCxGC and steroids

Natural mixtures such as urine are the most challenging chromatographic samples because of the sheer number of dissolved compounds. It is well known that even high resolution GC cannot resolve all components of complex mixtures, and pretreatment is often necessary to achieve adequate resolution for many analytes. This is a particular problem for analytes at low concentrations, such as the steroids in a urine matrix.

The quality of CSIA results is especially susceptible to chromatographic overlaps. We have shown that overlaps of as little as 10% cause the accuracy of isotope ratios to degrade by significant levels [13]. Insidiously, this degradation in accuracy does not come with a concomitant degradation in precision, which is the more easily monitored analytical parameter [13]. This can be partially remedied by the use of curve-fitting, which we will employ in our software development. However, a preferable approach is to achieve reliable baseline separations of analytes on a routine basis, ideally with little or no pretreatment.

In recent years, two dimensional GC (GCxGC) has developed into a commercial technique but is still at a very early stage [1, 36-38]. In this technique, a conventional GC capillary column is interfaced to a second, shorter conventional GC column that is chosen to separate on the basis of chemical properties orthogonal to the first column. Several techniques are used to trap and introduce analyte onto the second column, typically involving cryogenic stages or precise valving. This method has not yet been applied to IRMS but holds great promise to fully purify compounds prior to combustion and analysis in IRMS. In addition to its ability to resolve any desired overlapping peaks, it can do so for most peaks in a single run. It therefore holds great promise for survey isotope studies to develop additional candidates as markers for doping, including novel exogenous compounds.

Figure 3 is a diagram taken from a recent paper on GCxGC analysis of fecal steroids, a matrix that is at least as challenging as urine. A conventional GC is modified to accommodate a transfer device to pulse sample into a second, shorter GC column. In the device of figure 1, the transfer device is a cryogenic trap. The first GC separates compounds over a span of 30 min or so, and the transfer device traps eluting compounds over a short period of a few seconds. The transfer device then rapidly loads samples onto the second GC column, which separates them in a few seconds. During that separation, the transfer device traps eluting compounds for another few seconds to be

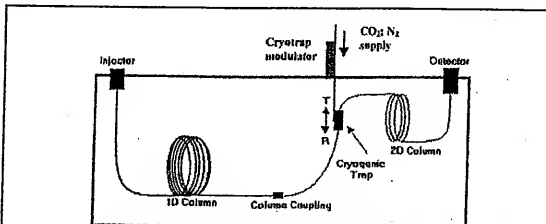


Fig. 1. Schematic diagram showing the GCxGC instrument design with a cryogenic trap located between the columns. For normal capillary GC operation, the modulation operation is not applied. Columns (chromatograms) 1 and 2 contain normal and fat column chromatograms, respectively.

Figure 3. Diagram of a GCxGC instrument used for steroid testing, from ref. [1].



loaded for the next run on the short column.

Results of analysis of fecal steroids are presented in figure 4. The overlaps that are present in the x-direction represent all non-resolved peaks in the first column. The separations in along the vertical axis, all over 4 seconds time, represent resolved overlaps. The blowup in the lower panel show a number of steroids that have been resolved in the second column. Because the goal of these analyses was resolution of as many components as possible, baseline resolution was not attempted for these steroids. We believe that judicious choice of columns would enable resolution of almost any small number of compounds, such as a series of steroids.

### c. Preliminary Data

#### Standards creation and use for CSIA

Our laboratory was among the first to create and publish a method for compound-specific isotope analysis standards [4]. The advent of commercially-available gas chromatography-combustion IRMS (GCC-IRMS) instruments in the late 1980s precipitated an interest in isotope standards of pure compounds, rather than the chemically ill-defined bulk standards available at that time, for instance, oil, polyethylene foil, or graphite [7].

Two methods are employed to calibrate isotope ratios in GCC/IRMS. The most common method is to admit a pulse of calibrated CO<sub>2</sub> gas from an independent volume during a period of flat baseline in the chromatogram. In this scheme, the calibrant does not pass through the GC or combustion interface, as does the sample, and has a substantially different peak shape. The alternative is to include a calibrated compound directly in the analysis mixture, as is routine for quantitative GC analysis. Such condensed-phase standards offer advantages such as obviating the requirement for gas handling apparatus. It has long been suspected that isotope ratios are sensitive to peak shape and integration parameters [39], however the convenience of introducing calibrated gas at any point in a chromatogram has led to the emergence of CO<sub>2</sub> from a volume as the most common means of introducing calibrant. Additional problems with this approach are that overlapping peaks can skew isotope ratio without affecting precision [13], a problem that can be rectified with curve-fitting.

In 1994 we reported a protocol for development of isotopically characterized standards useful for direct calibration of GCC-IRMS data and particularly useful for biomolecules which require derivatization prior to analysis [4]. Fatty acids are chosen as the model compound, and their corresponding methyl esters are calibrated to permit calculation of the isotope ratio of the target carbons. The principles and methods are extendable to any analytes and will form the basis of our methods for creating standards for steroids.

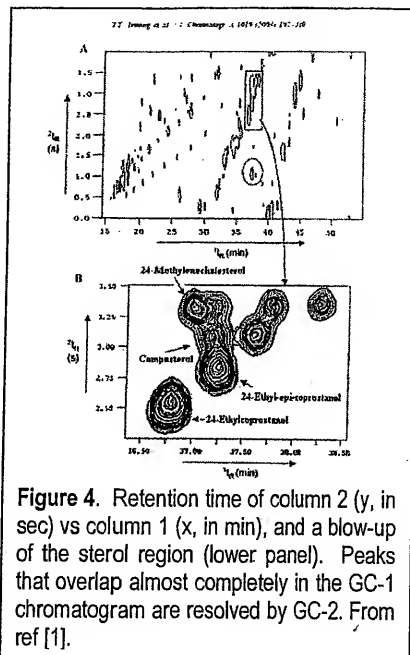


Figure 4. Retention time of column 2 (y, in sec) vs column 1 (x, in min), and a blow-up of the sterol region (lower panel). Peaks that overlap almost completely in the GC-1 chromatogram are resolved by GC-2. From ref [1].

The general strategy for creation of GCC/IRMS standards is to calibrate, by dual-inlet analysis, a large quantity of material, which can then be added to sample mixtures as an internal standard. It is critical that the calibrant be free of organic contaminants during bulk calibration since the calibrant will go through a separation step in routine use. When possible, standard compounds will have several desirable properties:

- (1) chemical stability against heat, light, and oxidation;
- (2) conveniently available in high purity;
- (3) soluble in high-purity solvents;
- (4) commonly used as standards for chromatography;
- (5) nonvolatile (i.e., very low vapor pressure) at room temperature and atmospheric pressure;
- (6) environmentally rare, to lower the probability of contamination;
- (7) useful for volatile (e.g., GC) and nonvolatile (e.g., Elemental Analyzer) introduction techniques.

These principles apply equally well to proficiency standards and working standards. We focus here on fatty acids, which are usually derivatized to their corresponding methyl esters prior to GC analysis. The carbon isotope ratio then is a weighted average of the target fatty acyl moiety and the methyl carbon. We choose two long-chain saturated fatty acids, heptadecanoic acid (17 carbons, 0 double bonds; "17:0"), heneicosanoic acid (21 carbons, 0 double bonds; "21:0"), and their corresponding methyl esters (Me17:0, Me21:0). These compounds are routinely used as internal standards for quantitative analysis of fatty acid profiles and are available at >99% purity from a variety of sources.

Reagents. Two grams of each of the four standards (purity >99%) were obtained from Matreya (Pleasant Gap, PA). Solvents used for dissolution of standards were Optima grade (Fisher Scientific). Solvent and standard purity was verified by capillary GC and used without further purification.

Isotopic Homogenization. It is well established that isotopic segregation is suspect at all phase changes, including precipitation from solution. Since most fatty acids are subjected to final purification by fractional crystallization, isotopic fractionation among crystals within a container must be assumed. To ensure isotopic homogeneity, crystalline standard was carefully placed in an acid-washed, pre-rinsed, all-glass dispensing vessel. Solvent was then added (hexane for methyl esters, chloroform for free fatty acids), and standard was allowed to dissolve completely at room temperature. From this point forward, care is taken to ensure that no evaporation occurs from this standard solution and that the solution is contained exclusively in acid-washed, pre-rinsed all-glass vessels, transfer lines, and flame seal vials. Solution containing 20-25 mg of standard (verified gravimetrically) was dispensed into acid-washed amber flame-seal vials. High-purity N<sub>2</sub> was used to evaporate solvent, leaving crystallized standard in the container. Care was taken to ensure that none of the solution splashed into the neck of the container during evaporation. Vials were then N<sub>2</sub>-flushed and flame-sealed.

Calibration. Vycor (Coming, Inc., Coming, NY) combustion tubes were prepared by flame-sealing one end, cleaning with solvent, and baking at 800°C for several hours. Five flame seal vials were chosen for analysis by use of a random number table. The outside of the vials was rinsed three times with high-purity solvent, cracked open, and placed in a dry pre-rinsed vessel. High-purity solvent was added, and the standard was permitted to dissolve completely with periodic gentle agitation. Solution containing about 3 mg of standard was pipetted into combustion tubes, and solvent was evaporated quantitatively using high-purity dry N<sub>2</sub>. CuO (200 mg) and Ag flake (25 mg) were added to the vials,

which were then evacuated to about  $10^{-5}$  Torr and flame-sealed. One blank was prepared by metering high-purity solvent into a combustion tube with identical processing steps. Standard samples of graphite obtained from the National Institute of Standards and Technology (NIST) designated "RN 8541" (USGS24<sup>a</sup>) were prepared in analogous fashion. For graphite, samples from a freshly opened container were carefully placed in separate combustion tubes using an acid-washed microfunnel instead of solvent to mediate transfer. A total of four tubes were prepared for the graphite standard. Combustion tubes were baked for 4 h at 800°C. The CO<sub>2</sub> gas from all combustion tubes was analyzed for carbon isotope ratio.

All CO<sub>2</sub> samples were analyzed with a Finnigan 252 IRMS instrument operated in dual-inlet mode. A single aliquot of CO<sub>2</sub> from a gas bottle was calibrated against the USGS24 standard value, reported by NIST as  $\delta^{13}\text{C} = -15.9\text{‰}$  ( $\pm 0.25\text{‰}$ ). All other tubes were referenced against this aliquot, and therefore, sample calibration can be traced to the NIST/USGS standard. The mass spectrometer was operating at a source voltage of 10 kV with a measured absolute sensitivity of about 800 molecules/ion detected.

**Carbon Isotope Ratio Results.** The isotope ratios determined by dual-inlet GIRMS analysis are presented in Table 1. Data from all tubes analyzed are presented; no outliers were eliminated. Four numbers are presented for 21:0 since one of the tubes exploded in the furnace during baking. The standard deviation estimates and 95% confidence limits (95% CL) reflect the total error associated with random isotopic variation among vials and all preparation steps including dissolution, loading into combustion tubes, and instrument errors. Internal reproducibility for the 252 instrument was  $\pm 0.05$  permil for each sample. The blank tube contained less than 0.5% of the CO<sub>2</sub> contained in the sample tubes, indicating negligible carbon contribution from reagents.

**Recommended Usage.** Because of considerations regarding fractionation at phase transitions, all standard in any vial must be dissolved completely in order to preserve the isotopic integrity of the overall sample. After complete dissolution, we refer to the overall standard as "reconstituted", and the carbon isotope ratio theoretically reflects that of the standard metered into each vial. Isolated crystals from a newly open vial cannot be certified to have the same isotope ratio as the overall container and may be expected to skew alter the isotope ratios of the entire vial if removed. Therefore, the following steps are essential to ensure the integrity of the standards upon first opening.

(1) Standards should be quantitatively dissolved in high purity solvent (hexane for methyl esters, chloroform for free fatty acids) and transferred quantitatively to a second large vessel. This will usually require two or more dissolutions and two final rinses to ensure complete transfer.

(2) From this second vessel, in which the standard solution is now reconstituted, the standard should be distributed into several containers. We recommend the use of both working containers and a set of flame seal vials for storage. ***Solvent must be evaporated completely prior to flame-sealing or an explosion may result.*** All vials should be rinsed with high purity solvent and dried before standard is transferred. Prior rinsing with high-purity solvents is essential to remove soluble contaminants; acid washing to remove traces of detergent is desirable.

Table 1

	17:0	Me17:0	21:0	Me21:0	USGS24 <sup>a</sup>
	-32.022	-31.144	-23.695	-27.957	-15.988
	-32.134	-30.833	-23.817	-27.936	-15.831
	-31.930	-30.840	-23.893	-27.828	-15.872
	-32.147	-30.876	-23.635	-27.885	-15.909
	-32.192	-30.905		-27.913	
mean	-32.085	-30.920	-23.760	-27.904	-15.900
SD	0.107	0.129	0.116	0.050	0.067
95% CL <sup>b</sup>	0.123	0.148	0.161	0.066	0.093

<sup>a</sup> Mean adjusted to accepted value of -15.900. <sup>b</sup> 95% confidence limits, calculated using Student's *t* distribution.



(3) Storage vials should be sealed under N<sub>2</sub> and stored at -20°C. Working vials should be tightly sealed using Teflonlined caps and stored in a cool place. The risk of contamination from environmental carbon is limited to those compounds that would be expected to elute simultaneously with the standard in a chromatographic analysis. This possibility is expected to be low, particularly since these compounds are relatively rare (e.g., absent in fingerprints and laboratory surfactants). A serious threat to the isotopic integrity is the possibility of fractional crystallization, which occurs when solutions are left loosely capped, and solvent evaporates and standard crystallizes on the interior of the container along the rim of the meniscus. When this occurs, crystals should be carefully redissolved.

Standards made according to this protocol were homogeneous in isotope ratio to about 0.1‰ from vial to vial. The protocol is general and extends to any desired compound of low volatility at room temperature. Upon request, a limited quantity of the standards reported here is available for distribution to CSIA laboratories for interlaboratory comparison and calibration.

#### d. Research Design and Methods

##### i. Standards creation

We will create 1) pure isotopically calibrated standards, 2) isotopically calibrated standards in urine matrix, and 3) pure isotopically calibrated internal working standards. Each will be used for different purposes according to the overall goals of Box 1 above.

*Standards in urine matrix for Proficiency and Round Robin.* Standards must be created in the urine matrix. Because all urine contains the target metabolites, it will be necessary to isotopically characterize metabolites (etio, adro,  $\alpha$ ,  $\beta$ ) within a stabilized urine pool, to be distributed to labs. This will be done by an isotope dilution strategy that we have employed in other contexts, which is an accurate, iterated version of the method of standard addition.

A single mixture of the four isotopically characterized pure standards will be prepared in saline from pure standards. Samples of the urine pool will be diluted gravimetrically in 5 increasing proportions (1:1, 2:1, ..., 5:1). Each sample will be isotopically characterized. A plot of isotope ratio vs. dilution yields the isotope ratio of the analytes in urine at zero dilution. Filtered and stabilized urine with the now isotopically characterized analytes will be distributed into vials, flame sealed, and distributed to labs.

*Preparation of pure standards* High and low isotope ratio standards for etio, adro,  $\alpha$ , and  $\beta$  will be created. Sources for standards with isotope ratios at the extremes, high and low, will be identified so that standards can be created at any desired level. There are several strategies for creating standards of low or high isotope ratio, among which are the ability to mix them later to obtain a standard of any desired isotope ratio. Pure samples will first be obtained from chemical supply vendors and other sources of pure steroids, and analyze the isotope ratios of batches of these compounds. Sources of extreme isotope ratios will be identified for all these compounds, and they will be obtained in large quantities of high and low isotope ratio. In this context, "large" quantity will be estimated as the amounts of standards that will be consumed as standards among the labs for tasks discussed in this work.

If the range of isotope ratios is insufficient for our purposes, we will intentionally fractionate standards by distillation. Isotope ratios are well-known to fractionate during changes in phase. Fractional crystallization does not always result in strong fractionation but since it does not require derivatization and requires only very simple apparatus, we attempt it first. Isotope ratios as a function of crystallization will be measured from a small sample. If fractionation is not successful, we will derivatize for volatility, and vaporize in appropriate all-glass apparatus. Evaporation is known to be a strong source of isotopic fractionation. Isotope ratio as a fraction of fractionation will be determined. Once a satisfactory source of isotopic fractionation is identified, large batches of standard and proceed will be prepared and isotopically calibrated.

These standards will be used for several purposes. They can be used as direct calibrants for GCC-IRMS by laboratories without the issues of matrix. They can be mixed according to any proportions with one another or with urine matrix to generate standards of any isotopic compositions. This is essential for proficiency standards that must be of unique and unknown composition during each round of proficiency testing.

Once identified, standards will be characterized according to the methods discussed in section c above. Briefly, multigram samples will be dissolved in appropriate solvent and secured in a manner to maintain isotopic integrity.

*Pure internal standards.* Internal standards will be created for addition to samples for isotopic calibration within a chromatogram. We understand that various Olympic testing labs are using various inhouse standards for particular analyses. We will first survey laboratories for this information and related information, including sample pre-treatment protocols, GC columns, temperature programs, and the like.

Internal standards added to sample mixtures prior to analysis must be designed so that they elute from the GC column in a flat region of the chromatogram. If labs are using a variety of extraction, derivatization, and GC conditions, it will not be possible to create a single standard that suits all conditions. We will evaluate the range of methods currently in use to determine whether a unique or small set of standards will suffice for most labs, and plan to create working standards accordingly. This will involve creation of standards with structures that will elute within the same chromatograms but not interfere (overlap) with any of the important analytes.

Once standards are created and calibrated according to methods established previously and outlined above, aliquots will carefully be dispensed into several hundred vials and flame sealed. Approximately 10 vials will be chosen at random, isotopically analyzed, and the spread in isotope ratios determined. The maximum tolerable variability in isotope ratio acceptable for GCC-IRMS analysis of real samples is  $SD(\delta^{13}C) < 0.30\%$  with no outliers excluded. Our variability will be below this figure and our aim will be  $SD(\delta^{13}C) < 0.15\%$  as we achieved previously. If this level is not achieved (as judged by a chi-squares test,  $p < 0.05$ ) we will discard the run of samples and remake them.

ii. Interlaboratory comparison, proficiency, and working standards.

Standards for Round Robin tests and calibration are equivalent and require that samples of unknown analyte isotope ratio can be easily created.



Round Robin. We will first conduct a Round Robin project to establish the range of variability among labs. We expect to provide three urine samples, calibrated as discussed above, (etio/andro or  $\alpha/\beta$ ) to all participating labs. Labs will have a specified time period, typically 2 weeks, to post coded results to a central website. Software will be created so that labs can log in to post results, and will post them with a private code for personal identification, but these codes will not be known to others. The Brenna group will calculate distributions and summary statistics and make them available along with private codes so that laboratories can identify themselves. This procedure can be repeated as many times as necessary given that the standards can be altered as desired to make different standards. Insofar as possible, we will attempt to add a component to these Round Robins not previously used in isotope studies, namely to have raw data files sent along with data. This would permit us to process all data on our single platform software, and determine whether software and/or overlaps are a major source of variability.

The information from this exercise will define the range of values that are obtained across various labs. The results of this test will help to define the magnitude of the harmonization problem, specifically the range of standards necessary to insure compliance and in part common deficiencies in expertise required to assist laboratories in bringing themselves up to expected performance. Study of the raw data will enable us to determine sources of variability in order to assist laboratories to improve analyses.

Proficiency Standards. In principle, the requirements for a Round Robin are equivalent to those for proficiency standards. Proficiency testing will require the organization of an authoritative body. We will be able to provide standards of novel isotope ratio, again generated from the calibrated standards available from the foregoing task.

Working Standards. Isotopically calibrated pure compounds for standard addition will be distributed to laboratories on request. Instructions on use will be developed and distributed, including specific use for common vendor supplied data reduction software.

Success in accomplishing tasks i and ii are essential to all underlined items in Box 1.

iii. Development of software for platform independent isotope measurement

We propose to develop software that runs independently of the vendor software and provides a common reporting format for steroid use. The software will load a chromatogram, identify the relevant peaks, integrate using either a conventional summation algorithm or a curve-fitting routine, and generate a report. The program is intended to be a common reporting platform and not a fix for acceptable chromatography.

The precise features of this code will depend on the chromatography used by the various labs. We anticipate that success in this aim may assist in standardization of methods. The program will be written in Visual Basic or LabView and run on Microsoft operating systems. We also have some curve-fitting and data-reduction routines, developed in-house, that can be adapted. It may be necessary for the vendors to cooperate in supplying data storage formats for our use. Software specifications will be developed with close consultation with USADA and other relevant experts.

#### iv. Analytical Chemical Research: GCxGC

The Brenna-laboratory routinely builds its own apparatus for isotope characterization upstream of the IRMS. We will implement the GCxGC system according to the same procedures used for other systems. Briefly, the output of the second GC will be directed to a rotary valve that can direct the flow to the combustion reactor or to the FID. The reactor is a 0.5mm ID x 0.125"OD x 20 cm ceramic tube filled with a braid of Cu/Ni/Pt wire. The furnace is a Thermcraft Fibercraft furnace operated at 950 C. The water trap is made from Nafion tubing and the open split is a simple T piece with a helium purge.

Our home-written software will be modified to control the GCxGC apparatus. It is set to control an output board with several relays for triggering events.

The major potential pitfall we anticipate in implementation is the rapid elution of peaks in GCxGC runs. The time constant for the 252 IRMS carbon isotope detectors is about 100 millisecc. Peaks in GCxGC can be shorter than 500 millisecc, thus challenging the IRMS to respond adequately. We will characterize the response of the IRMS to determine the minimum peak elution time that can yield adequate isotope ratios. There are two approaches to dealing with minimal response time issues. The first is to adjust chromatographic conditions so that peaks are sufficiently resolved in GC-2 to permit the peak to be sufficiently slow for proper response. This is a preferred solution because it requires only conventional adjustments to the system, in particular, flow rate. An alternative is to increase the response rate of the detectors by changing the properties of the amplification circuit. We have done this previously in the context of hydrogen analysis where response times for HD detection are an order of magnitude slower.

We will implement GCxGC according to methods appropriate for the main analytes. Methods will be refined to deliver acceptable accuracy and precision, and to improve analytical speed compared to current methods.

We believe this task is responsive to the call for additional research in the area, numbered item 6 in Box 1. It holds great promise for improving and extending sensitivity and specificity of doping analyses.

#### e. Plan for Result Transfer

We expect to work closely with USADA to refine initial goals for our research so that the outcomes are directly relevant to the recommendations of the Symposium (Box 1), and as may be modified since then. It will be most critical to work with USADA to establish authoritative testing and to engage as many labs worldwide as possible in our Round Robin(s) and in proficiency testing. With this in mind, the following are plans for result transfer.

#### i. Round Robin (Ring test) Results

The Round Robin test will include as many laboratories as are willing to participate. Each laboratory will have an opportunity to evaluate and refine their procedures based on results of their analyses compared to all others. As all results will be anonymous, the quality of the analyses will afford the labs a chance to both refine their techniques and to comment on the quality of our standards.

Results will first be circulated to participating labs. If results diverge by an unacceptable degree, we believe that it would be most fruitful to move into a refinement period rather than publishing results immediately. We then would conduct a second round Round Robin test to establish the degree of improvement and would expect to publish one or both sets of data. The decision of what and when to publish is expected to be in close consultation with USADA and the cooperating laboratories.

ii. Proficiency standards

A plan for establishing proficiency must be managed centrally by an authoritative body. We will work closely with USADA and its designees to develop a plan for proficiency qualification, as appropriate.

iii. Working standards

Together with USADA, we will make available working standards and guidelines for use to qualified Olympic Testing laboratories. Specific plans for invitations to laboratories to participate will depend on the timing of availability of standards and details to be worked out with USADA. Data and comments from a subset of laboratories will be collected and evaluated to refine instructions prior to general release to all interested parties.

Once established, distribution of working standards will be on a cost-recovery basis handled from the Brenna Lab at Cornell University. This will permit the central standards laboratory to maintain control over storage conditions and to create new standards as is warranted by demand. Should demand be outside Brenna Lab ability to sustain the service, we will seek to transfer distribution to one or more of several entities in the isotopic standards business, such as the Office of Standard Reference Materials of the US National Institutes of Standards and Technology in the Washington, DC area, or the International Atomic Energy Agency in Vienna.

iv. Software

The platform independent software to be developed under this program will eventually be refined in collaboration with interested Olympic testing laboratories and debugged for use by technicians with skills commensurate with operation of IRMS instrumentation. The ultimate use of the software will depend, as most items in this proposal, on recommendations of USADA.

v. Analytical Chemical Research GCxGC

Research results are best disseminated by presentation at appropriate conferences and by peer-reviewed publication. We have a strong record of bringing analytical advances to the scientific community via these means. We will also highlight our most important advances to USADA and to the user community so that they can be featured at symposia, as appropriate. Eventually, we envision training on novel techniques at our laboratory in Ithaca, NY if desired.



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g. Human Subjects

The only involvement of humans in this project is the use of human urine as a matrix for standards. It is our experience that human waste can be collected and used for research purposes under an exception from informed consent. We will seek an exception from the Cornell Institutional review Board on Human Subjects before initiating any use of human urine. If it is denied, we will seek full approval. **No work on human materials will be done without IRB approval.**

h. Vertebrate Animals

None.

**DETAILED BUDGET FOR INITIAL BUDGET PERIOD**

FROM

THROUGH

**DIRECT COSTS ONLY**

PERSONNEL (Applicant organization only)

DOLLAR AMOUNT REQUESTED (omit cents)

NAME	ROLE ON PROJECT	TYPE APPT (months)	% EFFORT ON PROJ.	INST. BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTALS
J. Thomas Brenna	Principal Investigator	9.0	20.0%	\$150,000	\$33,333	\$16,620	\$49,953
Gavin L. Sacks	Research Associate	12.0	100.0%	\$65,000	\$65,000	\$32,409	\$97,409
GRA	Res Asst	12.0	100.0%	\$38,000	\$44,121		\$44,121
SUBTOTALS					\$142,454	\$49,029	\$191,483

## EQUIPMENT (itemize)

Thermo Finnigan 253 IRMS, \$280,000  
GCxGC, 48,000

\$328,000

## SUPPLIES (itemize by category)

Pure compounds for standards creation, \$5000.  
High resolution GC columns and supplies (septa, liners, etc), high purity gases, \$4000.  
General lab supplies (reagents, solvents, vessels, etc), \$3000.  
GC maintenance and repair, \$2000.  
International carbon isotope standards, \$1000.

\$15,000

## TRAVEL

Project meetings and conferences, 10/yr, \$20,000

\$20,000

## OTHER EXPENSES (itemize by category)

Instrument maintenance

**SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD**

(Item 5a, Face Page)

\$ 554,483

INDIRECT COSTS -  
FACILITIES AND

Can not exceed 25% of direct costs (enter as a dollar amount)

\$138,621

**TOTAL COSTS FOR INITIAL BUDGET PERIOD**

(Item 5b, Face Page) →

\$ 693,104

**BUDGET FOR ENTIRE PROPOSED PERIOD OF SUPPORT**  
**DIRECT COSTS ONLY**

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD	ADDITIONAL YEARS OF SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL: <i>Salary and fringe benefits</i> <i>Applicant organization only</i>		\$191,483	\$199,143	\$207,108		
CONSULTANT COSTS						
EQUIPMENT		\$328,000				
SUPPLIES		\$15,000	\$15,600	\$16,224		
TRAVEL		\$20,000	\$20,800	\$21,632		
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES			\$20,000	\$20,800		
SUBTOTAL DIRECT COSTS		\$554,483	\$255,543	\$265,764		
TOTAL DIRECT COSTS for Entire Period		<b>\$1,075,790</b>				
INDIRECT COSTS	Can not exceed 25% of direct costs, enter as a dollar amount					
		\$138,621	\$63,885	\$66,441		
SUBTOTAL COSTS		\$693,104	\$319,428	\$332,205		
TOTAL COSTS FOR ENTIRE PROPOSED PERIOD OF SUPPORT			(Item 6b, Face Page)	→	\$	<b>1,344,737</b>

**JUSTIFICATION:****Personnel**

J. T. Brenna, the Principal Investigator, will be responsible for overall planning, execution, and reporting for the project. The PI will travel to sites, as appropriate, for research and project meetings. The PI has budgeted two months of summer salary to concentrate on meeting the goals of the proposed work.

Gavin L. Sacks, (PhD,) the Senior Research Associate, will be responsible for all day to day activities for the project. He will assemble and establish detailed protocols for standards, plan and execute logistics for round robins, plan and write code for the platform-independent software for reporting, and conduct all research related to the GCxGC project. He will also participate in report writing, including instructions for using software and for using standards.

A Graduate Research Assistant (GRA), to be identified, will assist the Dr Sacks in appropriate aspects of the project, including developing standards and executing research on GCxGC of steroids.

(continued)

## Budget Justification (continued)

## Equipment

IRMS. The Brenna Lab currently operates two isotope ratio mass spectrometers (IRMS), a Finnigan MAT 252 and a APP 2003 (a part of GV Instruments). The 252 is 15 years old and is operational for measurements for an average of 46-48 weeks per year over the last five years. This instrument was state-of-the-art in 1989 when purchased. The APP 2003 is 3 years old and is designed as a lower precision instrument; with home-built modifications it is enabled the APP to operate at similar precision to the 252. We have budgeted for a new state-of-the-art Thermo-Finnigan 253 IRMS, the current state-of-the-art IRMS which superceded the 252 several years ago. The 252 is suitable for all activities in this proposal. However, creation of standards for worldwide use, and especially in a legal context, may be more universally considered unassailable if calibrated by a new state-of-the-art system installed for the purpose. For this reason, we budget for a new instrument.

GCxGC. Components of a commercial system are budgeted to support the research project. The cost requested includes a switching system and software.

## Supplies

Pure compounds for standards creation, \$5000.

These compounds are to be calibrated and eventually distributed to the user community.

High resolution GC columns and supplies (septa, liners, etc), high purity gases, \$4000.

High resolution GC columns are \$500-1000 each and we anticipate requiring several to work out separations for standards and for GCxGC.

General lab supplies (reagents, solvents, vessels, etc), \$3000.

GC maintenance and repair, \$2000.

International carbon isotope standards, \$1000.

## Travel

We anticipate travel to periodically meet with USADA personnel for planning and reporting purposes, and to advisory meetings for assembling standards, and to Olympic testing laboratories worldwide to work out details for standards. We also expect to widely disseminate results of our round robin and our GCxGC research at conferences and symposia. The budget is for 10 journeys per year total, averaging \$2000 per journey.

We generally maintain our instruments with a graduate research assistant (GRA) plus equipment costs. We have budgeted less than half the total estimated amount of \$50,000 per year. For context, a manufacturer service contract for a 252 IRMS is roughly \$35,000 per year, with limitations replacement equipment (pumps, etc). Without a service contract, onsite service is billed as \$3,000 per day. If a new 253 is purchased, no service contract will be necessary until year 2, as is represented in the budget. If not, we will request \$20,000 for year 1 maintenance.

## OTHER SUPPORT

NAME OF INDIVIDUAL: J. THOMAS BRENNNA

### ACTIVE/PENDING (includes outstanding applications):

Title of Project (or Subproject): Gas Phase Derivatization for Lipidomic MS Analysis (ACTIVE)

Source (Organization and Identifying Code): NIH R01 GM71534

Dates of Approved/Proposed Project: 8/1/04-7/31/08

Annual Direct Costs: \$150,000

Total Direct Costs: \$957,033

Percent Effort (PI): 20%

The major goals of this project are aimed at development of novel ion-molecule reactions amenable to complete and high throughput characterization of lipids.

### OVERLAP

There is no scientific overlap between R01 GM71534 (Brenna) and the application under consideration.

Title of Project (or Subproject): Precise Isotope Ratio Chromatography and Stable Tracers (ACTIVE)

Source (Organization and Identifying Code): NIH R01 GM049209

Dates of Approved/Proposed Project: 7/1/01-6/30/06; currently in no-cost extension year.

Annual Direct Costs: \$175,000

Total Direct Costs: \$1,079,709

Percent Effort (PI): 15%

The major goals of this project are to develop high precision isotope ratio mass spectrometry for biomedical applications. The current grant period focuses on natural isotope ratios as tracers.

### OVERLAP

There is no scientific overlap between R01 GM049209 (Brenna) and the application under consideration.

Title of Project (or Subproject): Dietary Influence on Function (ACTIVE)

Source (Organization and Identifying Code): Mead-Johnson Nutritionals

Dates of Approved/Proposed Project: 11/15/02-3/21/06

Annual Direct Costs: \$244,058

Total Direct Costs: \$637,890

Percent Effort (PI): 10%

This project investigates the efficacy of the dietary polyunsaturated docosahexaenoic acid for development of the neonate baboon central nervous system.

### OVERLAP

There is no scientific overlap between this and the application under consideration.

Title of Project (or Subproject): Piglet Tissue Fatty Acids and DHA Supplementation (ACTIVE)

Source (Organization and Identifying Code): Martek Biosciences, Inc.

Dates of Approved/Proposed Project: 4/15/05-4/14/06

Annual Direct Costs: \$125,584

Total Direct Costs: \$198,422

Percent Effort (PI): 5%

This project investigates the efficacy of various single cell oils for supplying DHA and ARA to the brain and other tissues of growing piglets.

### OVERLAP

There is no scientific overlap between this and the application under consideration.

Title of Project (or Subproject): Dietary Influence on Function (PENDING)

Source (Organization and Identifying Code): Mead-Johnson

Dates of Approved/Proposed Project: 11/1/05-10/31/06

Annual Direct Costs: \$45,856

Total Direct Costs: \$104,216

Percent Effort (PI): 10%

This proposal is a supplement to the Mead-Johnson grant above to extend it for additional data analysis. The "percent effort" overlaps entirely with "Dietary Influence on Function" above.

#### OVERLAP

There is no scientific overlap between this and the application under consideration.

Title of Project (or Subproject): LCPUFA Status and Birth Outcome in India (PENDING)

Source (Organization and Identifying Code): NIH R03 HD052138-01

Dates of Approved/Proposed Project: 7/1/05-6/30/07

Annual Direct Costs: \$79,000

Total Direct Costs: \$158,000

Percent Effort (PI): 5%

The major goals of this project are to investigate the relationship of polyunsaturated fatty acids and birth parameters in a poor population in India.

#### OVERLAP

There is no scientific overlap between this and the application under consideration.

### RESOURCES/SAMPLE

#### **FACILITIES:**

**MAJOR EQUIPMENT:** The Brenna lab operates 3 molecular mass spectrometers and 2 isotope ratio mass spectrometers in the 1000 sq ft lab mentioned above. Two high precision isotope ratio mass spectrometers (Finnigan 252 and GV-APP 2003) with home-built GC-combustion inlets are also operated by the BrennaLab and are fully available to the project. A ABI-MDS-Sciex QTRAP 2000 triple quadrupole/linear ion trap mass spectrometer and an Applied Biosystems/Sciex API 3+ triple quadrupole mass spectrometer equipped with APCI and ESI sources, and homebuilt nano-spray source are fully available to the project for lipidomics analysis. A Varian Saturn 2000 ion trap mass spectrometer with integral GC and autoinjector is dedicated to the project, and a second Varian QISMS research ion trap is also available if necessary.

**Laboratory:** 1000 sq ft of wet laboratories including several hoods and another 1000 sq ft of dry lab space for instrumentation are dedicated to the PI's program. All ancillary laboratory equipment (centrifuges, balances, etc.) are in place and functioning on a daily basis.

**Clinical:** N/A

**Animal:** N/A

**Computer:** The Brenna laboratory operates no fewer than 16 PCs for instrumentation control, data analysis, and word processing. Most of the computers are linked to one another via a local network and to the Cornell IT system. Cornell University maintains extensive online library resources, a theory center with supercomputing capability, and inexpensive site licenses for many titles of scientific software.

**Office:** Adjacent to the lab are 700 sq ft of office space for research group members, and a 200 ft<sup>2</sup> office for the PI.

**Other:** A fully equipped physics machine shop, and physics and chemistry stockrooms are a 2 minute walk from the main lab at Cornell. An electronics shop is adjacent to the stockroom.



Principal Investigator/Program Director (Last, first, middle): Brenna, J. Thomas

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PERFORMANCE SITES

ORGANIZATION Cornell University  
CITY Ithaca  
STATE New York

Department: Division of Nutritional Sciences

KEY PERSONNEL

<u>Name</u>	<u>Organization</u>	<u>Role on Project</u>
J. Thomas Brenna	Cornell University	Principal Investigator
Gavin L. Sacks	Cornell University	Research Associate

**BIOGRAPHICAL SKETCH**

Provide the following information for the key personnel in the order listed on the previous page.  
Follow this format for each person.

NAME	POSITION TITLE		
J. Thomas Brenna	Professor and Director of Undergraduate Studies		
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
University of Connecticut	BS	1980	Nutritional Biochemistry
Cornell University	MS	1982	Chemistry (Analytical)
Cornell University	PhD	1985	Chemistry (Analytical)

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

- 1985-1989 Staff Chemist and Director, Fourier Transform Mass Spectrometry Laboratory, IBM Technology Laboratory, Endicott, NY
- 1989-1995 Assistant Professor, Division of Nutritional Sciences, Cornell University, Ithaca, NY
- 1995-2000 Associate Professor, Division of Nutritional Sciences, Cornell University, Ithaca, NY
- 2000-present Professor & Director of Undergraduate Studies, Division of Nutritional Sciences, Cornell University, Ithaca, NY
- 1994-present Member, Cornell Graduate Faculty of Chemistry and Chemical Biology.
- 1994-present Member, Cornell Graduate Faculty of Geological Sciences (subfield of Isotope Geochemistry).
- 2001-2009 Member, Board of Directors, Int. Soc. for the Study of Fatty Acids & Lipids (ISSFAL)
- 2000-2004 Member, Publications Committee, American Society for Mass Spectrometry (ASMS)
- 2002-present Editorial Advisory Board, Rapid Communications in Mass Spectrometry
- Selected Professional Societies [yr joined]: *Am Soc Mass Spectrom* [1986], *American Chemical Society* [1982], AAAS [1979], *ISSFAL* [charter member, 1990], *American Oil Chemists Society* [1979].

Federal Government Advisory Committee Membership:

- 2000-present, Member, Advisory Counsel, NIH Center for Biomedical Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA.

Recent Selected Review Service

- NIH: NCI Initial Review Group (IRG) (Jan 2004); NIH Roadmap IRG Metabolomics Technology Development (Jun 2004). IRG – Biological Chemistry and Macromolecular Biophysics (BCMB), 2002-2005 (10 rounds); 2002, NIH Initial Review Group (IRG) on Biological Mass Spectrometry; 1997, NIH Intramural Laboratory Review. NSF: Biological instrumentation Panel (June 2004). Various Private Foundations.

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- Carbon position-specific isotope analysis of alanine and phenylalanine analogues exhibiting nonideal pyrolytic fragmentation. Christopher J. Wolyniak, Gavin L. Sacks, Bruce S. Pan, J. T. Brenna. *Analytical Chemistry* 2005;77(6):1746-52
- On the Formation of Conjugated Linoleic Acid Diagnostic Ions With Acetonitrile Chemical Ionization Tandem Mass Spectrometry, Anthony L. Michaud, Peter Lawrence, Richard Adlof, J. Thomas Brenna, *Rapid Communications in Mass Spectrometry*, 19(3):363-368, 2005.
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- Sarkadi-Nagy E, Wijendran V, Diau G-Y, Chao AC, Hsieh AT, Turpeinen A, Nathanielsz PW, Brenna JT. The Influence of Prematurity and Long Chain Polyunsaturate Supplementation in Four-Week Adjusted Age Baboon Neonate Brain and Related Tissues. *Pediatric Res* 2003;54:244-252.
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- Brenna JT, Busch KL, Caprioli RM, Cotter RJ, Grigby RD, Judson CM, Ramanathan R, Siuzdak G, Story MS, Thomas JJ, Willoughby RC, Yergey AL (contributors). In: *Measuring Mass. From Positive Rays to Proteins*. M.A. Grayson, Ed. Sponsored by the Am. Soc. Mass Spectrometry 50<sup>th</sup> ASMS Conference, and distributed to all 4800 attendees. Published by Chemical Heritage Press, Philadelphia, 2002.
- Brenna JT. Efficiency of conversion of  $\alpha$ -linolenic acid to long chain n-3 fatty acids in man. *Curr Opin Clin Nutr Metabol Care* 2002; 5(2):127-132.
- Infante JP, Tschanz CL, Shaw N, Michaud AL, Lawrence P, Brenna JT. Straight-chain Acyl-CoA Oxidase Knockout Mouse Accumulates Extremely-Long-Chain Fatty Acids From  $\alpha$ -Linolenic Acid: Evidence for Runaway Carousel-type Enzyme Kinetics in Peroxisomal  $\beta$ -Oxidation Diseases. *Molec Genet Metab* 2002;75(2):108-119.
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- Brenna JT. Natural Intramolecular Isotope Measurements in Physiology. Elements of the Case for an Effort Toward High Precision Position-Specific Isotope Analysis (PSIA) *Rapid Comm in Mass Spec* 2001;15(15):1252-1262.
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- Su H-M, Corso TN, Nathanielsz PW, Brenna JT. Linoleic Acid Kinetics and Conversion to Arachidonic Acid in the Pregnant and Fetal Baboon *J Lipid Res* 1999;40(7):1304-1312.
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## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on the previous page.  
Follow this format for each person.

NAME Gavin L. Sacks		POSITION TITLE Post-Doctoral Associate	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
University of Virginia	BS	1999	Chemistry
Cornell University	MS	2001	Chemistry (Analytical)
Cornell University	PhD	2004	Chemistry (Analytical)

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

Professional Experience:

2001-2002 Consultant, Software Development, In Situ Isotope Services, Houston, TX  
2004-present Post-Doctoral Associate, Biocomplexity and Biogeochemistry Initiative (BBI), Cornell University, Ithaca, NY

Professional Societies [yr joined]: *Am Soc Mass Spectrom* [2001], *American Chemical Society* [1999],

Bibliography:

- Carbon Position-Specific Isotope Analysis of Alanine and Phenylalanine Analogues Exhibiting Nonideal Pyrolytic Fragmentation. C.J. Wolyniak, G.L. Sacks, B. Pan, and J.T. Brenna. *Analytical Chemistry*, 77(6), p.1746, 2005
- $^{15}\text{N}/^{14}\text{N}$  Position Specific Isotopic Analyses of Polynitrogenous Amino Acids. G.L. Sacks and J.T. Brenna. *Analytical Chemistry*, 77(4), p.1013, 2005
- Lipid Structure, Nomenclature, and Chemical Properties. J.T. Brenna and G.L. Sacks. Chapter 6 in *Nutritional Biochemistry*, 2<sup>nd</sup> Edition; M.H. Stipanuk, ed., 2005. [Textbook Chapter]
- High Precision Position Specific Isotope Analysis of  $^{13}\text{C}/^{12}\text{C}$  in Methionine and Leucine Analogues. G.L. Sacks and J.T. Brenna. *Analytical Chemistry*, 75(20), p.5495, 2003
- Analysis of Quantization Error in High Precision Continuous Flow Isotope Ratio Mass Spectrometry. G.L. Sacks, C.J. Wolyniak, and J.T. Brenna. *Journal of Chromatography A*, 1020(2), p.273, 2003
- Comparison of Microwave and Conventionally Heated Columns for Gas Chromatography of Fatty Acid Methyl Esters. G. Sacks and J.T. Brenna. *American Laboratory*, 35(18), p.22, 2003.
- Computational Modeling of Complexes of Penta-ammine Osmium(II) with Aromatic Ligands. C.O. Trindle, G. Sacks, and W.D. Harman. *International Journal of Quantum Chemistry*, 92, p.5, 2003

**Larry Bowers**

**From:** Makoto Ueki Ph.D [wd3m-uek@asahi-net.or.jp]  
**Sent:** Wednesday, February 01, 2006 9:43 PM  
**To:** Rabin, Olivier; Larry Bowers; david.cowan@kcl.ac.uk; Christiane Ayotte Ph. D.; Costas Georgakopoulos; Ray.Kazlauskas@measurement.gov.au; p.j.hemmersbach@farmasi.uio.no; moutian wu; Martial Saugy; Makoto Ueki; Jordi Segura, ; Joachim Grosse; Francesco Botr. 7. 7; Direction@lndd.com; dcatlin@ucla.edu  
**Cc:** Ivanova, Victoria; Boghosian, Thierry  
**Subject:** RE: Isotope ratio reference materials

Dear folks,

As discussed here already, calibration of reference CO<sub>2</sub> gas is an initial and critical step in the measurement of isotope ratios, as it may affect absolute delta parameters. However, the bias or the uncertainty of the initial calibration would not affect detectability of doping when we validate the results based on the relative delta parameters such as the ratio and/or the difference.

Even if it is a case, we still need to be careful because our results could be a target for the objection to be raised by isotope specialists who are working on the absolute delta values, such as Geo-scientists, Food chemists etc.. Furthermore, we can miss something if we focus only on the relative values or if we do pay attention only to any cases that fulfill the statement in the TD of ISL i.e. "the ratio measured for the metabolite(s) is below -28· per mill based on the non-derivatized steroid". We could observe few Asian cases with the higher delta parameters relative to the Anglo-Saxons or to the Caucasians.

In our case, we do calibrate reference CO<sub>2</sub> against NIST CRM Limestone and (I trust) it is enough, but the calibration against the CRM is not always easy to the scientists if not the laboratory is equipped with gas-phase isotope analysis system. I have been told about the story on the difficulty of CO<sub>2</sub> calibration by several laboratories.

Injectable CRM for isotope ratio measurement such as alkane or any steroid with known delta value seems to be convenient for all of us, as we need simply to inject these to the system for calibrating IRMS. (Question: inject the CRM before or after combustion furnace?)

More practically, I would suggest seeking at least one or more internal standards with known delta parameters, such as -20 and -30· per mill. Such IS would be a powerful tool to compensate uncertainty of isotope ratio calibration between the laboratory because the IS are added to each individual analytes. In addition, we can monitor repeatability of IR measurements for each batch of analysis, variation between batch and/or the traceability of the measurements to other laboratories.

In my personal opinion, use of common IS with known delta parameter for CIR measurements is more helpful than the use of reference standard.

Makoto UEKI

At 06/02/02 04:25, Rabin, Olivier wrote:

Dea All,

I am pleased to see a reactive discussion around this issue, and hope that we will benefit from your active Input for both the preparation and the implementation of the proficiency testing and CRM preparation for GC/C/IRMS.

Regarding the revision of the documents, we have initiated a revision of the ISL and various discussions have already occurred within the Laboratory Committee. It is my intention to review a consolidated version of the newly drafted ISL at the next Lab Com meeting in March before circulating this document for comments to the anti-doping laboratories and a few selected stakeholders or contributors.

My objective would be to have a reviewed version to inform WADA ExCo in May and hopefully finalize the new version for approval in September 2006. I do hope that we will be in a position to review the Technical Documents as we move along with the ISL, and proposed the revised versions simultaneously for approval or shortly after the new ISL.

As you can imagine this is a tentative planning that we will endeavour to achieve, but may vary due to interfering priorities at the level of the WADA ExCo.

I hope this information is helpful.

With best regards,

Olivier

**Dr. Olivier RABIN**

Director, Sciences

World Anti-Doping Agency / Agence Mondiale Antidopage

Tel: + 1 514 904 8829

Fax: + 1 514 904 8769

E-mail: [olivier.rabin@wada-ama.org](mailto:olivier.rabin@wada-ama.org)

Web: [www.wada-ama.org](http://www.wada-ama.org)

---

**From:** Larry Bowers [<mailto:lbowers@usantidoping.org>]

**Sent:** Wednesday, February 01, 2006 1:35 PM

**To:** david.cowan@kcl.ac.uk; Christiane Ayotte Ph. D.; Rabin, Olivier; Costas Georgakopoulos; Ray.Kazlauskas@measurement.gov.au; p.j.hemmersbach@farmasli.uio.no; moutian wu; Martial Saugy; Makoto Ueki; Jordi Segura; Joachim Grosse; Francesco Botr. 7. 7; Direction@Indd.com; dcatlin@ucla.edu

**Subject:** Isotope ratio reference materials

2/2/2007

USADA 1050

Colleagues,

In a continuing effort to assist in harmonization of test results among the laboratories, USADA has funded a research grant to both provide several isotopically certified reference steroids and will be working with WADA to develop characterized educational round robin/proficiency testing materials for GC/C/IRMS. To ensure that the program is fit for purpose, WADA and USADA will jointly appoint a Working Group consisting of three laboratory directors as well as two outside experts in GC/C/IRMS. The outside experts who have agreed to serve are Tom Brenna and Wolfram Meier-Augenstein. If you have an interest in being a member of this group, please let me know. We will make appointments by the end of February, and the group will probably meet for the first time in April. The research grant also aims to develop instrument-independent software that will use exponentially-modified Gaussian curve fitting for integration and reporting of data. < br> The ultimate utility of the reference materials should be determined by the Working Group with input from other laboratory directors with experience with GC/C/IRMS. I don't know whether an absolute delta value can be achieved, but with one or two common standards, we may be able to tighten the criteria somewhat.

With regard to the endogenous steroid Technical Document, following my experience with the Montgomery and Gaines non-analytical positive cases where this document came under significant scrutiny, I am of the opinion that it needs a major re-writing. As Christiane points out, the words that are used are very important, and one must consider all of the potential applications of the document in crafting it. I would hope that we would have both scientific and legal advice on its final form. I will not speak for Olivier with regard to how or when this task will be addressed.

Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
Office: (719)-785-2003  
FAX: (719)-785-2029

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\*\*\*\*\*

Makoto Ueki Ph.D Director/Anti-Doping Center  
Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.  
WADA Accredited Tokyo Lab.Japan

2/2/2007

USADA 1051

ISO17025 Accredited Toxicology Lab. (NATA No.14243)  
ISO15189 Accredited Medical Lab. (JAB:RML00020)  
CAP Accredited Toxicology and Hematology Lab.  
ISO9001 Certified Lab. (JCQA-0814)  
ISO14001 Certified Lab. (JCQA-E-0405)

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Tel: +81-3-5994-2351 Fax: +81-3-5994-2990

- Virus def updated 20 Jan, 2006 -

- Spyware def updated 18 Jan, 2006 -

\*\*\*\*\*



**Larry Bowers**

**From:** Kazlauskas, Ray [Ray.Kazlauskas@measurement.gov.au]  
**Sent:** Wednesday, February 01, 2006 10:11 PM  
**To:** Makoto Ueki Ph.D.; Rabin, Olivier; Larry Bowers; david.cowan@kcl.ac.uk; Christiane Ayotte Ph. D.; Costas Georgakopoulos; p.j.hemmersbach@farmasi.uio.no; moutian wu; Martial Saugy; Jordi Segura; ; Joachim Grosse; Francesco Botr????; Direction@Indd.com; dcatlin@ucla.edu  
**Cc:** Ivanova, Victoria; Boghosian, Thierry  
**Subject:** RE: Isotope ratio reference materials

We have our CO2 calibrated in a similar way to Makoto and it is done by a group at CSIRO external to ourselves. we obtained two large cylinders which should last us a few years. We do not observe any of the fractionation Makoto reported with his CO2. We have a supply of internal standards such as 5 $\alpha$ -androstanol, methyltestosterone which we have measured by combustion and values are very similar to what we obtain by IRMS. We monitor these in every run to ensure all is OK. We have also a small amount of substances such as pregnanediol, 11-ketotestosterone, androsterone and etiocholanolone (commercial suppliers) and may try to get others which we are about to submit for combustion analysis to get accurate CIR values (at CSIRO) these are directly compared to the international standard. These we will check on our instrument and I hope on some others as well and I hope to provide some to all labs with IRMS in June through WAADS. While these may be useful in preparing standards for controls and ongoing checking of data (control charts?) there are many more that could be useful and obtaining really well certified standards should be the overall aim. These need to be certified as to purity as well as the absolute CIR value. That is a difficult and expensive project. An even more difficult project would be preparation of a certified reference material, a urine with all relevant compounds having well defined values to be run with each batch. This would allow correction or a better understanding of our bias.

I agree with others that the best measurement is the difference to the ERC. This probably should be the only criterion once the ranges are validated in each lab. The absolute value is limited by population studies and does not reflect the real situation in an individual. However to get accurate differences the absolute values need to be measured well (error is additive for difference not relative so is large) which means we need a handle on how well our data is calibrated. Thus the TDs need to be overhauled for this reason.

Ray

---

**From:** Makoto Ueki Ph.D [mailto:wd3m-uek@asahi-net.or.jp]  
**Sent:** Thursday, February 02, 2006 3:43 PM  
**To:** Rabin, Olivier; Larry Bowers; david.cowan@kcl.ac.uk; Christiane Ayotte Ph. D.; Costas Georgakopoulos; Kazlauskas, Ray; p.j.hemmersbach@farmasi.uio.no; moutian wu; Martial Saugy; Makoto Ueki; Jordi Segura; ; Joachim Grosse; Francesco Botr. 7. 7; Direction@Indd.com; dcatlin@ucla.edu  
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**Subject:** RE: Isotope ratio reference materials

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Director, Sciences

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E-mail: [olivier.rabin@wada-ama.org](mailto:olivier.rabin@wada-ama.org)

Web: [www.wada-ama.org](http://www.wada-ama.org)

---

**From:** Larry Bowers [<mailto:lbowers@usantidoping.org>]

**Sent:** Wednesday, February 01, 2006 1:35 PM

**To:** david.cowan@kcl.ac.uk; Christiane Ayotte Ph. D.; Rabin, Olivier; Costas Georgakopoulos; Ray.Kazlauskas@measurement.gov.au; p.j.hemmersbach@farmasi.uio.no; moutian wu; Martial Saugy; Makoto Ueki; Jordi Segura; Joachim Grosse; Francesco Botr. 7. 7;

**Direction@Indd.com; dcatlin@ucla.edu**

**Subject:** Isotope ratio reference materials

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Larry

Larry D. Bowers, Ph.D.  
 Senior Managing Director  
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3-30-1 Shimura, Itabashi-ku, Tokyo 174-8555, JAPAN  
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 - Virus def updated 20 Jan, 2006 -  
 - Spyware def updated 18 Jan, 2006 -

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The Commonwealth does not warrant that any attachments are free from viruses or any other defects. You assume all liability for any loss, damage or other consequences which may arise from opening or using the attachments.  
\*\*\*\*\*

**Larry Bowers**

---

**From:** Christiane Ayotte Ph. D. [christiane.ayotte@laf.lhrs.ca]  
**Sent:** Wednesday, February 01, 2006 8:49 AM  
**To:** david.cowan@kcl.ac.uk; 'Rabin, Olivier'; 'Costas Georgakopoulos'; Ray.Kazlauskas@measurement.gov.au; p.j.hemmersbach@farmasi.uio.no; 'moutian wu'; 'Martial Saugy'; 'Makoto Ueki'; Larry Bowers; 'Jordi Segura'; 'Joachim Grosse'; 'Francesco Botrj\$!\$'; Direction@lndd.com; dcatlin@ucla.edu  
**Subject:** RE : Service Asked

Dear David,

Obviously, we will have to go through and clarify the interpretation of the technical documents and note.

As far as I can speak for my colleagues who wrote the TD2004EAAS, our intent was not to suggest relying solely on absolute IRMS delta values. On the contrary. However, reading again the document, I understand the interpretation one can make of the last phrase of that paragraph which you quoted: 'The results of such analyses will be reported as "inconclusive" unless the ratio measured for the metabolite(s) is below -28% based on the non-derivatised steroid.' Our intent was to not report simply as inconclusive a result when clearly depleted values were measured for the metabolites. A couple of words are missing: the criteria for reporting an adverse finding are not met but the testing authority should be informed that it is a highly suspicious test result which should not be simply considered as negative. We could document or explain to the testing authority what we mean.

I am personally not in favour of reporting on absolute values uniquely.

So let's make sure we can correct that interpretation in a next version and bring the necessary improvements needed. For example, I noticed that we totally forgot female athletes' values in the criteria for conducting an IRMS (200 ng of testosterone being a bit high...).

We had two interesting cases lately, borderline steroid profiles both giving clearly depleted delta values (Case A: androsterone and etio around 10,000/12,000 ng/mL, DHEA G+ F = 190 ng/mL, sulphate: 1,200 ng/mL and normal T/E value: IRMS values depleted for A, etio, DHEA; Case B: sp. gravity: 1,023; T/E = 2,6, T: 105 ng/mL, Andro: 11,300, Etio: 2,200 (A/Etio 5 – that's what triggered the IRMS) – IRMS values depleted for Andro and Etio).

Cordially,

Christiane

Christiane Ayotte, Ph.D.  
Laboratoire de contrôle du dopage  
INRS-Institut Armand-Frappier

2/5/2007

—Message d'origine—

**De :** David Cowan [mailto:david.a.cowan@kcl.ac.uk]

**Envoyé :** 1 février 2006 08:06

**À :** Rabin, Olivier; Costas Georgakopoulos; Ray.Kazlauskas@measurement.gov.au;  
p.j.hemmersbach@farmasi.uio.no; moutian wu; Martial Saugy; Makoto Ueki; Larry Bowers; Jordi Segura;;  
Joachim Grosse; Francesco Botrè; Direction@Indd.com; dcatlin@ucla.edu; David.A.Cowan; Ayotte,  
Christiane

**Cc :** Larry Bowers

**Objet :** RE: Service Asked

Dear Olivier

This is good news but I thought that Costas was referring to certified reference materials that we could use for traceability purposes. The work by LGC (LGC/VAM/2000/037) indicates that it is important to use standards run through the GC rather than relying on calibrating the CO2 alone if one is to be able to use absolute delta units as required in TD2004EAAS which I state here for clarity 'The results of such analyses will be reported as "Inconclusive" unless the ratio measured for the metabolite(s) is below -28% based on the non-derivatised steroid.'

Can you clarify please?

Regards

David

Professor David Cowan  
Head of Department of Forensic Science & Drug Monitoring  
Director, Drug Control Centre  
King's College London  
150 Stamford Street  
London SE1 9NH  
Tel: 020-7848 4848  
Fax: 020-7848 4980

—Original Message—

**From:** Rabin, Olivier [mailto:Olivier.Rabin@wada-ama.org]

**Sent:** 31 January 2006 19:31

**To:** Costas Georgakopoulos; Ray.Kazlauskas@measurement.gov.au;  
p.j.hemmersbach@farmasi.uio.no; moutian wu; Martial Saugy; Makoto Ueki; Larry Bowers; Jordi  
Segura;; Joachim Grosse; Francesco Botrè; Direction@Indd.com; dcatlin@ucla.edu;  
David.A.Cowan; Christiane Ayotte

**Cc:** Larry Bowers

**Subject:** RE: Service Asked

Dear All,

In response to Costas's message below, please be informed that USADA and WADA are currently collaborating to develop some GC/C/IRMS reference materials and prepare for a round robin proficiency testing program.

You should receive more information on the weeks to come on this program actively piloted by USADA following the meeting on IRMS held

2/5/2007

USADA 1059

in California a little more than a year ago.

All the best,

Olivier

**Dr. Olivier RABIN**

Director, Sciences

World Anti-Doping Agency / Agence Mondiale Antidopage

Tel: + 1 514 904 8829

Fax: + 1 514 904 8769

E-mail: [olivier.rabin@wada-ama.org](mailto:olivier.rabin@wada-ama.org)

Web: [www.wada-ama.org](http://www.wada-ama.org)

---

**From:** Costas Georgakopoulos [<mailto:oaka@ath.forthnet.gr>]

**Sent:** Wednesday, January 04, 2006 2:52 AM

**To:** Ray.Kazlauskas@measurement.gov.au; p.j.hemmersbach@farmasli.uio.no; Rabin, Olivier; moutian wu; Martial Saugy; Makoto Ueki; Larry Bowers; Jordi Segura; Joachim Grosse; Francesco Botrè; [Direction@Indd.com](mailto:Direction@Indd.com); [dcatin@ucla.edu](mailto:dcatin@ucla.edu); David.A.Cowan; Christiane Ayotte

**Subject:** Fw: Service Asked

Dear friends,

I wish you and your families health and success for the new year.

Is anybody interested for steroids with reference 5 value ?

Costas

Costas Georgakopoulos Ph.D

Director

Doping Control Laboratory of Athens

OAKA, Kifissias 37

15123, Maroussi, Greece

T: +30-210-6834567

F: +30-210-6834021

E: [oaka@ath.forthnet.gr](mailto:oaka@ath.forthnet.gr)

----- Original Message -----

**From:** Jon E. Johansen

**To:** [oaka@ath.forthnet.gr](mailto:oaka@ath.forthnet.gr)

**Sent:** Wednesday, December 21, 2005 11:38 AM

**Subject:** VS: Service Asked

Dear Dr Georgakopoulos,

I refer to your e-mail of December 7. I am sorry for my late reply, I was in bed with a flu and the left the message.

Anyway, we are very interested in cooperating with you.

As a company we are mostly interested in certifying products which can be supplied by Chiron afterwards.

2/5/2007

USADA 1060



If you let us know which steroid you plan to use, we could certify the quality and then make a common batch of this and offer this for the community.  
Please let us know if this is of interest.

Kind regards  
Dr. Jon E. Johansen  
Chiron AS  
Stiklestadveien 1  
N-7041 Trondheim

-----Opprinnelig melding-----

**Fra:** Gina E. Strom [mailto:gina.strom@chiron.no]  
**Sendt:** 7. desember 2005 15:35  
**Til:** Jon Johansen  
**Emne:** VS: Service Asked

-----Opprinnelig melding-----

**Fra:** Costas Georgakopoulos [mailto:oaka@ath.forthnet.gr]  
**Sendt:** 7. desember 2005 15:42  
**Til:** Chiron@chiron.no  
**Emne:** Service Asked

Dear Sir or Madam,

As it is listed in your online catalogue (pdf), you provide various alkanes in cyclohexane solutions for isotopic ratio measurements. These alkanes come with a certificate of their delta value for carbon. Since our applications involve steroid analysis, I would like to ask you if it is possible to arrange for a certification by Chiron of the delta values (i.e. carbon 13 and oxygen 18) for a steroid that we will provide you with. This means that we could send you a sample (e.g. vial) of the steroid solution, and you could then perform any necessary analysis and certify its delta value.  
If the above is possible, please inform me about its cost and the time that it will take.

Thank you very much in advance.

Yours sincerely,

Costas Georgakopoulos Ph.D  
Director  
Doping Control Laboratory of Athens  
OAKA, Kifissias 37  
15123, Maroussi, Greece  
T: +30-210-6834567  
F: +30-210-6834021  
E: oaka@ath.forthnet.gr

2/5/2007

**Larry Bowers**

---

**From:** Larry Bowers  
**Sent:** Tuesday, March 07, 2006 6:58 AM  
**To:** Tom Brenna  
**Subject:** Cologne Workshop

Tom,  
Hopefully you received the invitation for the Cologne Workshop. Andrea Gotzmann, who is the primary organizer, informed me that the IRMS session will probably be on Wednesday, June 7. She also suggested that we have the initial GC/C/IRMS Working Group Meeting on Wednesday late in the day. You and I will need to work on an agenda for the meeting and distribute it prior to the meeting.

You will need to register for the meeting on-line as described in the invitation. I would suggest that you plan to arrive on Sunday, as the opening reception is a good place to meet and greet people. My suggestion would be to stay at the Queen's Hotel (a Holiday Inn) on Durener Strasse in Cologne, which is where I have my reservation. Unfortunately I do not arrive (from Tokyo) until about 9 PM, so I will miss the reception. It's about a 25 min walk to the Institute, but it is a reasonably nice hotel, and the walk is through a very nice park. They usually also have a bus for the less motivated. You can get to Cologne by air or by train if you fly into Frankfurt, although as I remember it, the train ride is about 2.5 hours through a relatively pretty river valley.

Let me know if I can be of further assistance.  
Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
Office: (719)-785-2003  
FAX: (719)-785-2029

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2/5/2007

USADA 1062

**Larry Bowers**

**From:** Larry Bowers  
**Sent:** Wednesday, March 08, 2006 9:57 AM  
**To:** 'w.meier-augenstein@qub.ac.uk'  
**Subject:** RE: USADA GC/C/IRMS Working Group

Wolfram,  
Yes, Brenna's grant was funded. But we also wanted to start a working group to harmonize laboratory performance even before Brenna's grant. Tom will be at the meeting in Cologne. Please put it on your calendar. I will be in touch in a few days to fill in travel and hotel plans.

Thanks for your willingness to participate.  
Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
Office: (719)-785-2003  
FAX: (719)-785-2029

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**From:** Dr W Meier-Augenstein [mailto:w.meier-augenstein@qub.ac.uk]  
**Sent:** Wednesday, March 08, 2006 1:59 AM  
**To:** Larry Bowers  
**Subject:** RE: USADA GC/C/IRMS Working Group

Dear Larry,

Thank you very much for your e-mail.

At present this day is free in my diary (and so is the day after since I can't see how this could be done as a day return trip), so, by all means, count me in.

Let me check flight / train connections and time tables though, so I can give you a better, i.e. more definite answer. May I assume if an overnight stay in Cologne or en route is required, this will also come under the heading of travel expenses?

Just only realising the implication of what you have said, I take it this meeting means Tom Brenna's grant application to USADA has been successful?

Best regards,

Wolfram

|-----Original Message-----

2/5/2007

**From:** Larry Bowers [mailto:lbowers@usantidoping.org]  
**Sent:** 07 March 2006 16:51  
**To:** w.meler-augenstein@qub.ac.uk  
**Subject:** USADA GC/C/IRMS Working Group

Wolfram,

I am trying to arrange the first meeting of the GC/C/IRMS Working Group. Could you come to Cologne on the 7<sup>th</sup> of June for a late afternoon meeting? Tom Brenna is able to come and we are doing the meeting in Cologne because many of the laboratory directors will be there. USADA will cover your travel expense.  
Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
Office: (719)-785-2003  
FAX: (719)-785-2029

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2/5/2007

USADA 1064

**Larry Bowers**

**From:** Larry Bowers  
**Sent:** Wednesday, March 22, 2006 10:15 AM  
**To:** 'andrea.gotzmann'  
**Cc:** Elsha Symanski  
**Subject:** RE: Cologne Workshop

Thanks for your response. I will assume that I do not need to submit the BALCO topic in order to be considered for the program. I will probably submit an abstract for inclusion in the materials to be distributed.  
Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
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**From:** andrea.gotzmann [mailto:andrea@biochem.dshs-koeln.de]  
**Sent:** Wednesday, March 22, 2006 1:32 AM  
**To:** Larry Bowers  
**Subject:** Re: Cologne Workshop

Larry,

thank you for your message. It is no problem to start the IRMS meeting Wednesday from 6 or 6:30 pm. We will make reservation for a conference room at the Trainerakademie, means food and drinks are also available. The participants will miss the evening program, but there is always a next Workshop in Cologne III

Please add Willi Schaezler (instead of Hans Geyer) on your list for the meeting.

The abstracts will be distributed to all participants, but it is your decision whether to give some written information about the BALCO case or not.

Regards  
Andrea

\*\*\*\*\*  
Dr. Andrea Gotzmann  
Institut fuer Biochemie  
Deutsche Sporthochschule Koeln  
Carl-Diem-Weg 6  
D 50833 Koeln  
Tel +49 221 4982 4910  
Fax +49 221 497 32 36  
[www.dopinginfo.de](http://www.dopinginfo.de)  
\*\*\*\*\*

2/5/2007

— Original Message —

From: Larry Bowers

To: [andrea@blochem.dshs-koeln.de](mailto:andrea@blochem.dshs-koeln.de)

Sent: Tuesday, March 21, 2006 8:17 PM

Subject: Cologne Workshop

Andrea,

My registration has been received. Would you like me to submit something on the BALCO case under your call for papers?

It appears that Meler-Augenstein cannot get to Cologne until about 6 PM based on flights from Belfast. I would prefer that he be present for the whole meeting, if possible. If we started the GC/C/IRMS meeting at 6 PM, I presume that we would miss the evening's entertainment. Our meeting would tie up Beghosian, me, Brenna, Geyer, Kazlauskas, Ueki, and someone from UCLA. I want to be sure that we do not cause problems for the meeting, but also to get the work of the group completed. I need to get back to Meler-Augenstein as soon as possible.

Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
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2/5/2007

USADA 1066

**Larry Bowers**

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**From:** Elisha Symanski  
**Sent:** Friday, May 26, 2006 2:22 PM  
**To:** Thierry Boghosian (thierry.boghosian@wada-ama.org); Larry Bowers; schaezner@blochem.dshs-koeln.de; Makoto Ueki Ph.D; graham.trout@agal.gov.au; w.meier-augenstein@qub.ac.uk; jtb4@cornell.edu; bahrens@ucla.edu  
**Cc:** msekera@ucla.edu  
**Subject:** GC-C-IRMS Working Group  
**Attachments:** Agenda.doc

Attendees,

We look forward to working with you as a part of the GC-C-IRMS Working Group. We will be meeting on Wednesday, June 7, 2006 at the Trainerakademie at 6pm. Thanks again for being available for this meeting. I have attached a summary of discussion points for the working group to discuss. In preparation for this meeting, please bring with you your copy of the *Application of GC-C-IRMS to Doping Control* monograph from the 2<sup>nd</sup> Annual USADA Symposium on Anti-Doping Science. If you have any questions about the meeting, please do not hesitate to contact me.

*Sincerely,  
Elisha*

*Elisha Symanski  
U.S. Anti-Doping Agency  
1330 Quail Lake Loop, Suite 260  
Colorado Springs, CO 80906  
719-785-2023 phone  
866-601-2632 toll-free  
719-785-2001 fax*

2/5/2007

USADA 1067

**GC-C-IRMS Working Group**

Wednesday, June 7, 2006

Trainerakademie – 6pm

**Cologne, Germany**

<b>Attendees:</b>	Larry Bowers	(US Anti-Doping Agency)
	Thierry Boghosian	(World Anti-Doping Agency)
	Wilhelm Schänzer	(WADA Laboratory – Cologne, Germany)
	Makoto Ueki	(WADA Laboratory - Japan)
	Graham Trout	(WADA Laboratory - Australia)
	Brian Ahrens	(WADA Laboratory – Los Angeles, USA)
	Wolfram Meier-Augenstein	(Queens University Belfast)
	Thomas Brenna	(Cornell University)

Our focus should be on the scientific reliability of the GC-C-IRMS technique in establishing, or ruling out, the administration of a prohibited substance. Summarizing the key conclusions of the USADA Annual Symposium on GC/C/IRMS, the following issues require further development and resolution. The discussion points for the June '06 Meeting will be:

- I. Discuss the role of peak asymmetry, resolution, and peak fitting software
- II. Brainstorm the steps need to be taken to develop a Reference Material and/or Internal Standard material to assist the laboratories in achieving more uniform results
- III. Brainstorm the steps need to be taken to develop a Proficiency Testing Round Robin to improve harmonization of reported values
- IV. Determine what guidelines should be used: assay characteristics and performance criteria that will unify the methods in doping control
- V. Determine if there is benefit in considering the absolute  $\delta^{13}\text{C}$  values compared to the reference ranges of individual target analytes

**Future Working Group Meeting Discussion Points:**

- VI. Provide recommendations on the combination of longitudinal testing and GC-C-IRMS to provide cost-effective testing strategies: A GC-C-IRMS may be required in every case of an elevated T/E ratio
- VII. Discuss doping agents or techniques that could increase testosterone concentrations while maintaining a natural  $^{13}\text{C}/^{12}\text{C}$  ratio
- VIII. Discuss if GC-C-IRMS measurements are always definitive for an exogenous administration of testosterone or its precursors
- IX. Discuss if GC-C-IRMS replaces some of the other analytical tools for doping analysis or does it complement them
- X. Determine specific assay improvements that are needed
- XI. Determine what recommendations should be made to WADA



**Larry Bowers**

---

**From:** Makoto Ueki [wd3m-uek@asahi-net.or.jp]  
**Sent:** Tuesday, August 31, 2004 4:43 AM  
**To:** Larry Bowers; wd3m-uek@asahi-net.or.jp  
**Cc:** Myself  
**Subject:** Re: ISTD for CIR/MS

Dear Larry,

In my opinion, best way to compensate inter-laboratory deviation of isotope ratio measurements is a use of common internal standard with known delta values as internal standard is added to all analytes.

Actually, however, IS being added is not unified yet. According to the response to the latest CIR/MS survey, only 5 out of 11 participating laboratories are using any of 5 ISTDs i.e., 5alpha-androstan-3beta-ol, Octacosane, Androstanol, Methyltestosterone, 17alpha-methylandrostandiol.

In addition to these compounds, we can use Squalene or Hexatriacontane, and former elute near 5alpha-androstan-3beta-ol and later elute near free testosterone. Delta of our Squalene is about -20 and that of hexatriacontane is -28.5. Further, most of plant origin steroids do have delta of about -30. They correspond to the values for human steroids, cut-off and positive samples respectively.

One difficulty is that commercially available CRM are not steroid and not directly applicable to daily work. We are calibrating CIR/MS using in-house calibrated CO2, and the CO2 is periodically calibrated by CO2 gas generated from Limestone CRM from NIST. We have checked in-cooperation with Christians and Willi alkane mixture with known delta values from Indiana and confirmed its applicability. The Indiana reference is not CRM but is widely accepted by geo-scientists.

I think that we do not have to add ISD to compensate inter-laboratory deviation into the sample being confirmed at the first step in the analysis because CIR measurement is almost isotope dilution technique. If there is any extraction problem, then we only need to add the ISTD just prior to the sample injection. We would only need to be consider the chromatographic separation.

I wish my comments would be of any help to you.

At 04/08/25 23:09, Larry Bowers wrote:

>  
>Makoto,  
>I am in the process of reviewing the monograph from the GCC IRMS meeting.  
>In one of the discussions, there is a comment that you are running  
>"internal standards" in your samples as opposed to endogenous reference  
>compounds. Would you be willing to share what the internal standard  
>compounds are? USADA is looking into having a reference material(s)  
>for GCC IRMS prepared.  
>Larry  
>  
>Larry D. Bowers, Ph.D.<?xml:namespace prefix = o ns =  
>"urn:schemas-microsoft-com:office:office" />  
>  
>US Anti-Doping Agency  
>  
>2550 Tenderfoot Hill Street, Suite 200  
>  
>Colorado Springs, CO 80906-7346  
>  
>lbowers@usantidoping.org  
>  
>(719)-785-2003  
>

>(719)-785-2001 (FAX)

>

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Makoto Ueki Ph.D Director/Doping Control Laboratory

Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.

IOC/WADA Accredited Tokyo Laboratory

ISO17025 Accredited Toxicology Lab. (NATA No.14243)

CAP Accredited Toxicology and Hematology Lab.

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- Virus Checker Updated 9th. Aug, 2004 -

- Spyware Checker Updated 10th. Aug, 2004 -

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**Larry Bowers**

---

**From:** Tom Brenna [tbrenna@gmail.com]  
**Sent:** Wednesday, November 29, 2006 2:50 PM  
**To:** Larry Bowers  
**Subject:** Re:

Hi Larry,

I'm here and 10 am is good. 607-255-9182

Quick update:

- 1) The 253 is almost installed, as of last week.
- 2) We have nine of the standards preliminarily calibrated, and as soon as the 253 is ready, we'll do it for real and expect to have some standards in time for Cologne.
- 3) We have implemented the world first (we think) fast GCC-IRMS. 600 millisec wide peaks (FWHH), and we also think we can reduce analysis times to 25% of conventional. This is a necessary prelude to GCxGCC-IRMS, but I'm imagine it might be of interest in its own right.

I hope to have the standards and fast GC in time for Cologne.

Tom

On 11/29/06, Larry Bowers <[lbowers@usantidoping.org](mailto:lbowers@usantidoping.org)> wrote:

Tom,

Are you around? I would like to discuss where things are with the grant work. Also, WADA has funded Georgakopoulos and Kazlauskas for a GC/C/IRMS project. We should discuss this and see if there is a way to work together. I will plan to call you Thursday, Nov 30 at around 10 AM your time unless I hear otherwise.

Larry

Larry D. Bowers, Ph.D.

Senior Managing Director

U.S. Anti-Doping Agency

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2/5/2007

USADA 1072

**Larry Bowers**

---

**From:** Tom Brenna [tbrenna@gmail.com]  
**Sent:** Thursday, November 30, 2006 10:37 AM  
**To:** Larry Bowers  
**Subject:** Re: Email contacts

Larry,

Thanks for the follow-up.

Regarding instrument modifications, the changes are minimal and should not affect any other aspect of the analysis. We just swapped out a resistor in the m/z 46 detector to reduce the time constant (responds quicker). It can easily be done on a Delta, the conventional instrument that the other labs have. Frankly, the m/z 46 signal doesn't have much impact on the isotope ratios anyway, and we might not need it. It is used to correct the 45/44 ratio for 17O (12C17O16O). This is important for natural CO2 because the 17O could have any value but it is superfluous for GCC runs calibrated with an internal standard because almost all the oxygen in the CO2 has the same isotope ratio, thus assigning a d13C to a 45/44 ratio defines it completely.

Regarding software, we've not worked a lot more on that mostly because it's in good enough shape to be beta-tested for general purposes, but we'd also like to customize for a set of analyses that will be routine, and we don't exactly know what those conditions will be. Also, you may have heard that Thermo bought out GV this summer, so there is effectively a single manufacturer of IRMS. We all expect the software to converge now, so it would be useful to give them a little time before we reinvent more wheels. And yes, we're actually analyzing the fast GC data with the software now.

Tom

On 11/30/06, Larry Bowers <[lbowers@usantidoping.org](mailto:lbowers@usantidoping.org)> wrote:

: Tom,

: Good talking to you this morning. Sorry I was a bit late.

: The email addresses are:

: Costas Georgakopoulos (Greece): [oaka@ath.forthnet.gr](mailto:oaka@ath.forthnet.gr)

: Moutian Wu (China): [moutianwu@public.bta.net.cn](mailto:moutianwu@public.bta.net.cn)

: Makoto Ueki (Japan): [wd3m-ueki@asahi-net.or.jp](mailto:wd3m-ueki@asahi-net.or.jp)

2/5/2007

Congratulations on the progress ! This is pretty exciting. One thought that I had was that since the labs mostly have the routine GC/C/IRMS instruments as opposed to a 252 or 253, is there potential to make similar modifications to that instrument? What is the impact on the "validation" of the instrument?

We didn't talk at all about the software, but you may want to think about that in terms both of fast GC on the 252 and the "other" instruments that are out there.

Larry

Larry D. Bowers, Ph.D.

Senior Managing Director

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USADA 1074

**Larry Bowers**

---

**From:** Larry Bowers  
**Sent:** Friday, December 01, 2006 10:26 AM  
**To:** 'jtb4@cornell.edu'  
**Subject:** RE: SYDNEY-ATHENS IRMS project and USADA IRMS project  
**Attachments:** WADA IRMS ATHENS SYDNEY TO USADA 061114.pdf

Your email hit here about the same time that I dug out a copy of the proposal. Let me know what you think.  
Larry

Larry D. Bowers, Ph.D.  
Senior Managing Director  
U.S. Anti-Doping Agency  
Office: (719)-785-2003  
FAX: (719)-785-2029

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**From:** Tom Brenna [mailto:tbrenna@gmail.com]  
**Sent:** Friday, December 01, 2006 10:13 AM  
**To:** Costas Georgakopoulos; Ray.Kazlauskas@measurement.gov.au  
**Cc:** Larry Bowers; Mazzoni, Irene; Rabin, Olivier  
**Subject:** Re: SYDNEY-ATHENS IRMS project and USADA IRMS project

Dear Costa and Ray,

Good to hear that things are moving forward with you. It would be helpful to see an outline of your plans so to harmonize our work, insofar as possible. All material confidential, of course.

We are in the midst of calibrations and of other progress along the lines discussed in Cologne, and a few other matters that may improve steroid isotope analysis. In a few weeks we'll be in a position to share initial results and I'm expecting to have some isotopically calibrated standards for late February.

With regards,  
Tom

On 11/30/06, Larry Bowers <[lbowers@usantidoping.org](mailto:lbowers@usantidoping.org)> wrote:

Costas,

I spoke with Tom Brenna today. He has made considerable progress since last March, and has a variety of steroids with various delta values. We have also discussed a potential educational WADA PT sample for the first half of 2007.

I think collaboration is important. To contribute to that, I have copied Tom on this email so that you have his

2/5/2007

contact information. Please keep me in the loop.

We will plan to have another GC/C/IRMS working group meeting during the Cologne Workshop. I think we need to have good conversations now because eventually I would hope that we could reduce the number of standards and ERCs to one or two so that the Inter-lab agreement is not a dispute at hearings.

Larry

Larry D. Bowers, Ph.D.

Senior Managing Director

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Office: (719)-785-2003

FAX: (719)-785-2029

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---

**From:** Costas Georgakopoulos [mailto:[caka@ath.forthnet.gr](mailto:caka@ath.forthnet.gr)]

**Sent:** Tuesday, November 14, 2006 1:51 AM

**To:** Larry Bowers

**Cc:** Mazzoni, Irene; 'Rabin, Olivier'; [Ray.Kazlauskas@measurement.gov.au](mailto:Ray.Kazlauskas@measurement.gov.au)

**Subject:** SYDNEY-ATHENS WADA RESEARCH PROJECT ON IRMS

Dear friends,

I hope all be healthy and in good mood.

I'm attaching the recent approved WADA research project, submitted by Sydney and Athens, on the IRMS, proposing a different calibration procedure for the  $\delta$  values. We believe, that this procedure, in combination with the use of common to all labs RMs with known  $\delta$  values, will improve accuracy between WADA labs.

So Larry, since USADA also invests a severe amount and effort to the improvement of the IRMS measurements, any collaboration between our teams would be valuable. I believe for us the availability of RMs to build  $\delta$  value calibrations is first priority.

2/5/2007



Looking forward to your input and comments,

best wishes

Costas

Costas Georgakopoulos, Ph.D  
Director  
Doping Control Laboratory of Athens  
OAKA, Kifissias 37, 15123, Maroussi, Greece  
t: +30-210-6834567  
f: +30-210-6834021  
e: [oaka@ath.forthnet.gr](mailto:oaka@ath.forthnet.gr)

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2/5/2007

USADA 1077

## APPLICANT DECLARATION

Name: Floyd A. Landis  
Annual License#: 20272  
RACING AGE (as of December 31, 2006): 31  
Address: 23356 BISHOP RD  
City: MURRIETA ST: CA ZIP: 92562  
Phone: (909) 461-8462

Additional representations for International and USPRO Licenses

I am not aware of any reason why the requested license should not be issued. I have not requested a license from the UCI or from any other National Federation for the calendar year. I am solely responsible for the information contained in this application to the UCI, its Continental Federations, and its National Federations. I agree that the sole jurisdiction for resolving disputes that may arise shall be in the courts of the domicile of the UCI. By participating in a race where a drug test is conducted, in accordance with UCI, World Anti-doping Association (WADA) or U.S. Anti-Doping Agency (USADA) drug test regulations, I agree to submit to such testing. I further agree that when competing outside the United States or in international races the results of the analysis may be released to the public and communicated to my trade team, coach, or doctor in accordance with UCI and WADA regulations. I agree to allow my doctor and/or the doctor of my team, upon the request of the UCI or WADA, to release to UCI and WADA officials a list of medications or treatments administered to me before any specific competition. I agree to submit any protests or disputes regarding drug testing when competing outside the United States or in international races to the Court of Arbitration for Sports (CAS), whose decision I shall accept as final. I agree that all urine samples in such cases taken shall become the property of the UCI and WADA, and that UCI and WADA may have them analyzed for any purpose, including, without limitation, general research and information on health protection. I accept these conditions regarding testing and agree to undergo all tests required of me.

Signature of Applicant

Date

## CONSENT AND AGREEMENT OF PARENT OR GUARDIAN

I am the parent or guardian of Floyd A. Landis (Child). I give permission for my Child to enter any event permitted or sanctioned by USA Cycling, another event national federations, or International Cycling Union (UCI) during the period of the license applied for. **I HAVE READ AND I UNDERSTAND THE ABOVE CONTRACT.** In consideration of allowing my Child to participate, I consent to the contract and agree that **ITS TERMS SHALL LIKEWISE BIND ME, MY CHILD, my heirs, legal representatives, and assignees.** I **HEREBY RELEASE AND SHALL DEFEND, INDEMNIFY AND HOLD HARMLESS THE RELEASEES FROM EVERY CLAIM AND ANY LIABILITY** that I or my Child may allege against the Releasees (including reasonable legal fees and costs) as a direct or indirect result of injury or death to me or my Child because of my Child's participation in a USA Cycling event, **WHETHER CAUSED BY THE NEGLIGENCE OF THE RELEASEES or others. I PROMISE NOT TO SUE RELEASEES** on my behalf or on behalf of my Child regarding any claim arising from my Child's participation in a USA Cycling event.

Signature of Parent or Guardian

Date

The above signed agreement should be returned to:

USA Cycling, Inc.  
ATTN Membership Department  
1 Olympic Plaza  
Colorado Springs, CO 80909

JAN 20 2006

Please note a minor's on-line application cannot be fully processed and the license cannot be issued until this signed agreement is received.

USADA 1078

<p class=normal align=center style=text-align:center><b>ACKNOWLEDGMENT  
OF RISK, RELEASE OF LIABILITY, INDEMNIFICATION AGREEMENT AND  
COVENANT NOT TO SUB</b></p>

<p class=normal><b>  
I ACKNOWLEDGE THAT BY SIGNING THIS DOCUMENT, I AM ASSUMING  
RISKS, AND AGREEING TO INDEMNIFY, NOT TO SUE AND  
RELEASE FROM LIABILITY USA CYCLING, INC. (USAC), ITS ASSOCIATIONS  
(THE UNITED STATES CYCLING FEDERATION (USCF);  
NATIONAL OFF ROAD BICYCLE ASSOCIATION (NORBA), NATIONAL  
COLLEGIATE CYCLING ASSOCIATION (NCCA), U.S. PROFESSIONAL  
RACING ASSOCIATION (USPRO), AND BMX ASSOCIATION (BMXA)), AND USA  
CYCLING DEVELOPMENT FOUNDATION (USACDF), AND  
THEIR RESPECTIVE AGENTS, EMPLOYEES, VOLUNTEERS, MEMBERS,  
SPONSORS, PROMOTERS AND AFFILIATES (COLLECTIVELY  
"RELEASEES"), AND THAT I AM GIVING UP SUBSTANTIAL LEGAL RIGHTS.  
THIS DOCUMENT IS A CONTRACT WITH LEGAL AND  
BINDING CONSEQUENCES. I HAVE READ IT CAREFULLY BEFORE SIGNING,  
AND I UNDERSTAND WHAT IT MEANS AND WHAT I AM  
AGREEING TO BY SIGNING.  
</b></p>

<p class=normal>  
In consideration of the issuance of a license to me by one or more of Releasees and being  
allowed to participate in an event permitted or sanctioned  
by USA Cycling, another national federation or International Cycling Union (UCI)  
(collectively &quot;USA Cycling event&quot;) I hereby freely agree to and make  
the following contractual representations and agreements. <b>I ACKNOWLEDGE  
THAT CYCLING IS AN INHERENTLY DANGEROUS SPORT AND  
FULLY REALIZE THE DANGERS OF PARTICIPATING IN AN EVENT</b>,  
whether as a rider, official, coach, mechanic or otherwise, and <b>FULLY  
ASSUME THE RISKS ASSOCIATED WITH SUCH PARTICIPATION  
INCLUDING</b>, by way of example, and not limitation: dangers associated with  
man made and natural jumps, the dangers of collision with pedestrians, vehicles, other  
riders, and fixed or moving objects; the dangers arising from  
surface hazards, including pot holes, equipment failure, inadequate safety equipment, use  
of equipment provided by the event organizer and others,  
<b>THE RELEASEES' OWN NEGLIGENCE</b>, the negligence of others and weather  
conditions; and the possibility of serious physical and/or mental  
trauma or injury, or death associated with events. For myself, my heirs, executors,  
administrators, legal representatives, assignees, and successors in  
interest (collectively "Successors") <b>I HEREBY WAIVE, RELEASE, DISCHARGE,  
HOLD HARMLESS, AND PROMISE TO INDEMNIFY AND NOT  
TO SUE</b> the Releasees and all sponsors, organizers, promoting organizations,  
property owners, law enforcement agencies, public entities, special

districts and properties that are in any manner connected with a USA Cycling event, and their respective agents, officials, and employees through or by which the event will be held, (the foregoing are also collectively deemed to be Releasees), <b>FROM ANY AND ALL RIGHTS AND CLAIMS INCLUDING CLAIMS ARISING FROM THE RELEASEES' OWN NEGLIGENCE</b>, which I have or which may hereafter accrue to me, and from any and all damages which may be sustained by me directly or indirectly in connection with, or arising out of, my participation in or association with a USA Cycling event, or travel to or return from a USA Cycling event; in which I may participate as a rider, team member, spectator, coach, mechanic, official, volunteer, or in any other manner. I agree it is my sole responsibility to be familiar with the course and agenda of a USA Cycling event, the Releasees' rules, and any special regulations for a USA Cycling event and agree to comply with all such rules and regulations, including, that I must submit to drug testing, if required. I understand and agree that situations may arise during a USA Cycling event which may be beyond the control of Releasees, and I must continually ride and otherwise participate so as to neither endanger myself nor others. I accept responsibility for the condition and adequacy of my equipment, any equipment provided for my use, and my conduct in connection with a USA Cycling event. I have no physical or medical condition which would endanger myself or others if I participate in a USA Cycling event, or would interfere with my ability to safely participate in a USA Cycling event. <p>  
<p class="normal align="center style="text-align:center"><b>APPLICANT DECLARATION</b></p><br>

<p class="normal align="left style="text-align:center"><b>Additional representations for International and USPRO Licenses</b></p>

<p class="normal">  
-----  
I am not aware of any reason why the requested license should not be issued. I have not requested a license from the International Cycling Union (UCI) or from any other national federation for the calendar year. I am solely responsible for the information contained in this application and for the use I shall make of the license. I shall undertake to respect and comply with the constitution and regulations of the UCI, its Continental Federations, and its National Federations. I agree to compete in a sporting manner. I agree that the sole jurisdiction for resolving disputes that may arise shall be in the courts of the domicile of the UCI. By participating in a race where a drug test is conducted, in accordance with UCI, World Anti-doping Association (WADA) or U.S. Anti-Doping Agency (USADA) drug test regulations, I agree to submit to such testing. I further agree that when competing outside the United States or in international races the results of the analysis may be released to the public and communicated to my trade team, coach, or doctor in accordance with UCI and WADA regulations. I agree to allow my doctor and/or the doctor of my team, upon the request of the UCI or WADA, to release to UCI

and WADA officials a list of medications or treatments administered to me before any specific competition. I agree to submit any protests or disputes regarding drug testing when competing outside the United States or in international races to the Court of Arbitration for Sports (CAS), whose decision I shall accept as final. I agree that all urine samples in such cases taken shall become the property of the UCI and WADA, and that UCI and WADA may have them analyzed for any purpose, including, without limitation, general research and information on health protection. I accept these conditions regarding testing and agree to undergo all tests required of me.

&lt;/p&gt;

&lt;p class=normal&gt;&lt;b&gt;Electronic Signature.&lt;/b&gt;

I have read this agreement and I understand and agree to be bound by its content. I understand that by clicking the below AGRBB button or by pressing any button on this web-site which causes the transfer of information for the purpose of ordering from this site or using it's services, establishes an agreement to the above Acknowledgement of Risk, Release of Liability, Indemnification Agreement and Covenant Not to Sue, and that such choice will constitute the equivalent of an Electronic Signature by the user, signifying an intent to fully abide by and agree to the above terms, making this a binding contractual agreement between USA Cycling and user. I understand by doing so that I have given up substantial rights.

&lt;/p&gt;

floydlandis - 2006-01-11 12:10&lt;br&gt;Accepted electronic waiver&lt;br&gt;&lt;br&gt;

URINE RESULTS FROM IMPROD LAB  
 10/6/2004/10/6

Bottle#	Collection	ISG	TE	Total	(Epid)	(Acid)	(Eto)	(GAD)	5HAT	DHEA
37038	04/30/00	N/A	2.40	N/A	N/A	N/A	N/A	N/A	N/A	N/A
336873	08/05/03	107	1.80	847	573	AT11	295	732	4960	20.00
342898	09/04/04	1019	1.70	253	145	170	1002	248	1053	21.83
342853	09/11/04	1012	2.00	73	37	395	435	59	184	17.63
342860	09/12/04	1025	1.40	393	294	2803	2702	324	2449	27.40
348368	09/13/04	1019	1.30	265	194	1646	1301	223	1337	15.40
37038	09/14/04	1015	5.00	362	373	2593	1591	927	2837	21.80

10/6/2004/10/6

(Neurotic & premenstrual)  
 (Panic for the momentary)

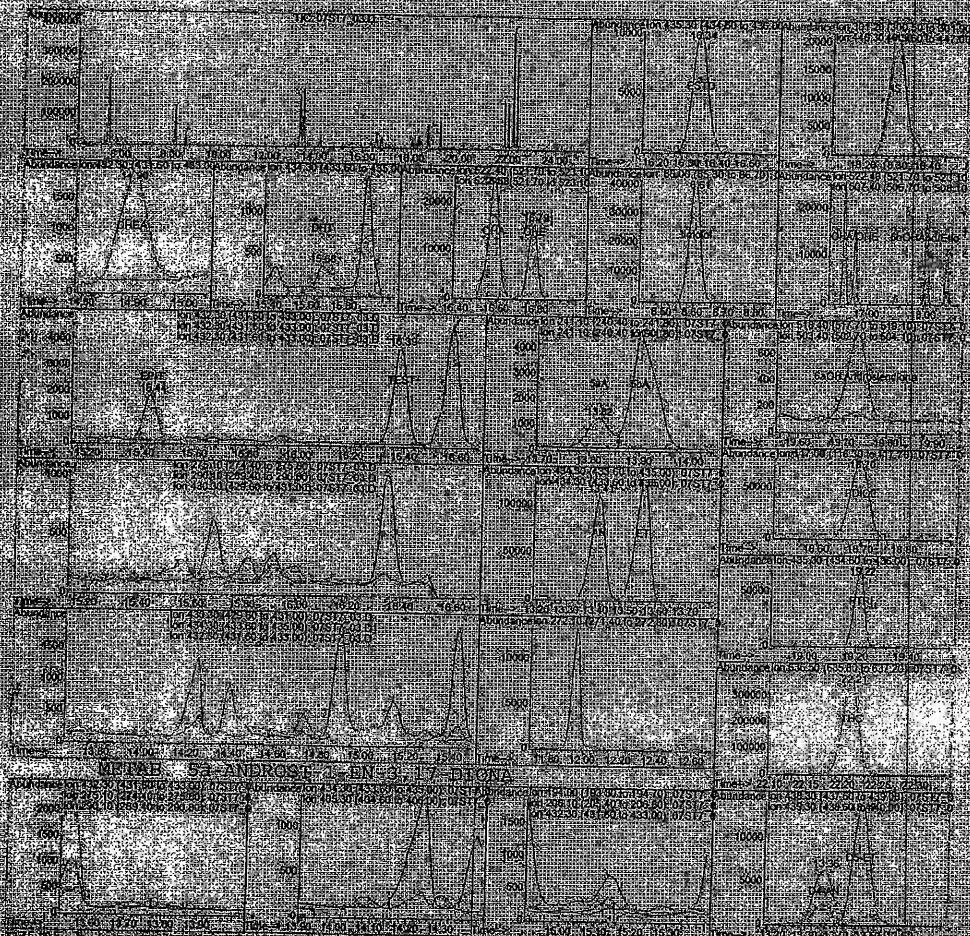
How much it takes to recover

THE





21.19	METYLTESTOSTERONA	133908	250		
10.87	PIMOLOL	19	119		
17.80	615-ANDROSTERONA	894871	2583		
10.02	BILICOLANOLONA	434610	1298		
15.39	5-ANDROL	14976	47.6	$[An]/[IT] =$	48.6
16.54	5B3ADIOL	46943	173.4	$[An]/[EpI] =$	76.1
19.93	DEBA	14967	30.2		
20.70	6H1TESTOSTERONA	19749	34.0	$[T]/[EpI] =$	2.8
21.11	DHT	2576	8.3	$[An]/[EpI] =$	2.0
33.06	TESTOSTERONA	84671	53.1	$[OH]/[OH] =$	0.9
22.18	OHA	418897	589.5		
33.71	OHE	66101	660.7	$[5aADIOL]/[5ADIOL] =$	0.3
34.53	PREGNANDIOL	230956	71.4	$[5ADIOL]/[EpI] =$	1.4
24.99	PREGNANTRIOL	21917	411.1	$[DHT]/[EpI] =$	6.4
32.96	TETRAHIDROCORTISOL	807020	1546.7	$[DHT]/[EpI] =$	0.2



LA RELACION metil/lincol: 0.40

LA RECUPERACION metil: 2.64

LA RECUPERACION lincol: 1.00

18-30 TESTOSTERONA

95076 260

18-31 TINGOL

108690 234

18-32 17-ANDROSTERONA

362380 1761

18-33 17-ANDROSTADIOL

420558 1802

18-34 17-ANDROSTADIOL

1937 27.9

18-35 17-ANDROSTADIOL

11584 141.9

18-36 17-ANDROSTADIOL

5493 21.6

18-37 17-ANDROSTADIOL

6253 12.5

18-38 17-ANDROSTADIOL

1508 8.3

18-39 17-ANDROSTADIOL

15023 25.8

18-40 17-ANDROSTADIOL

188328 382.7

18-41 17-ANDROSTADIOL

54806 387.4

18-42 17-ANDROSTADIOL

239716 187.6

18-43 17-ANDROSTADIOL

198225 1289.3

18-44 17-ANDROSTADIOL

598273 1781.7

18-45 17-ANDROSTADIOL

[An]/[T] = 68.3

[An]/[Epit] = 121.2

[T]/[Epit] = 1.9

[An]/[Epit] = 0.9

[OH]/[OH] = 1.5

[SADIOL]/[SADIOL] = 0.2

[SADIOL]/[Epit] = 1.9

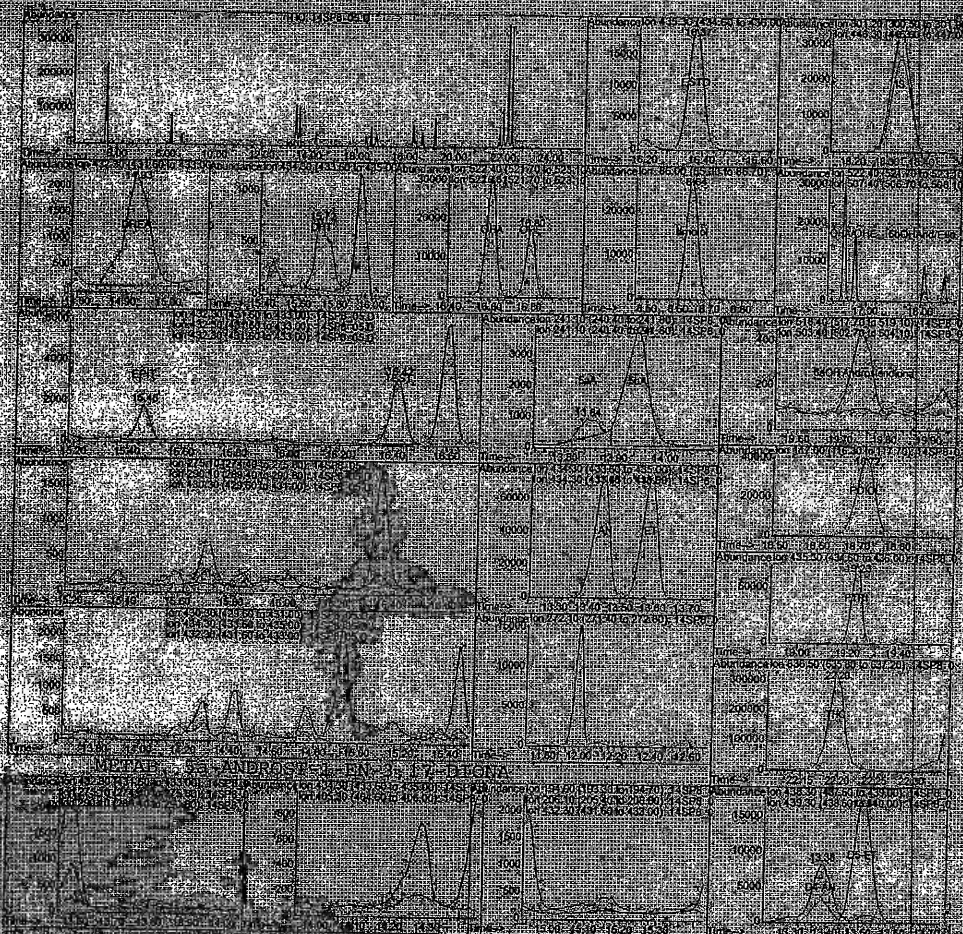
[OH]/[Epit] = 1.4

[OH]/[Epit] = 0.6



D:\1\TAREA 9 040811\143PS 05-D Sample 3-2736  
 MUESTRA:  
 POSICION Landa: 04127069A(0) 041219 VIAL: 5

14 Sep 2004 13:41  
 METODO: RTE-03.M



17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET  
 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET 17-ET

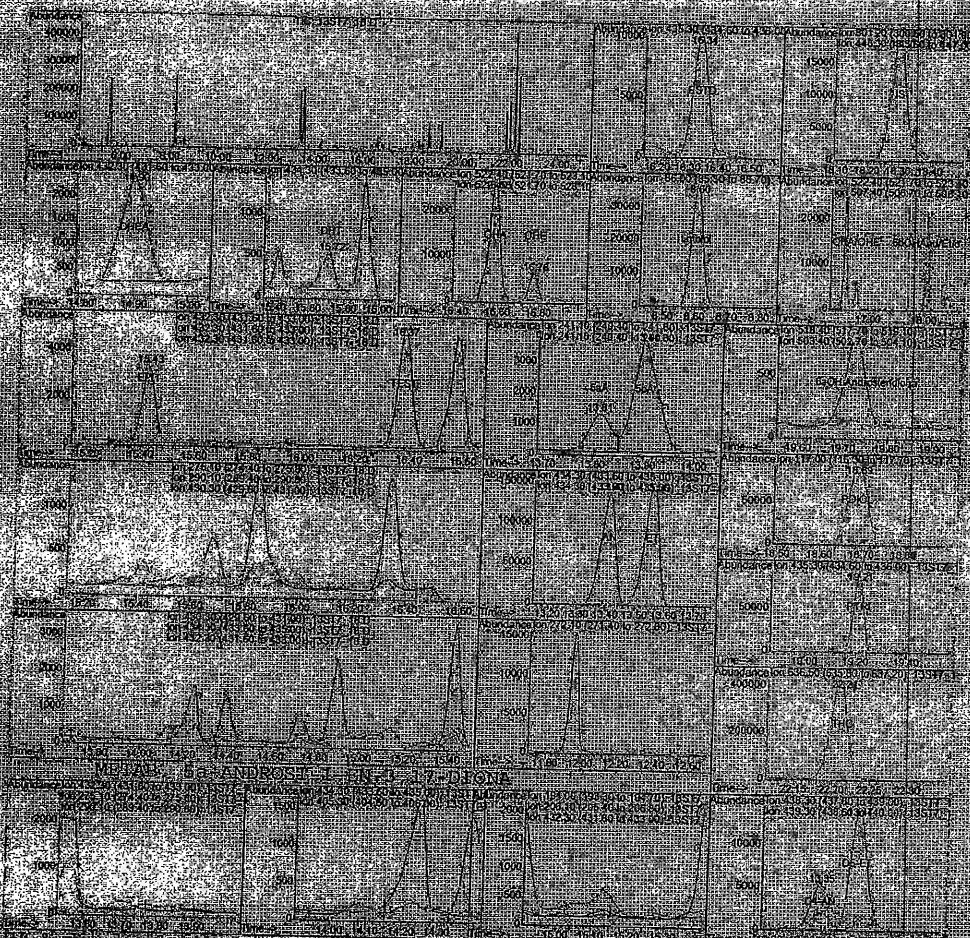
LA RELACION metil/limbol: 4.66  
 LA RECUPERACION metil: 1.48  
 LA RECUPERACION limbol: 0.89

13.33 METILTESTOSTERONA	121547	250
13.64 LIMBOL	73187	285
13.75 CAS-ANDROSTERONA	242082	1181
13.82 ET-ECOLANOLONA	238483	1200
13.84 5-ANDRIOL	1619	14.1
13.91 5-ANDRIOL	9553	152.0
14.03 DHEA	9113	29.4
15.16 EPI-TESTOSTERONA	5538	12.7
15.22 DHT	4041	23.5
16.12 TESTOSTERONA	12618	22.6
16.61 DHA	123407	375.7
16.80 DHB	58068	211.0
18.75 PREGNANDIOL	128813	110.6
19.21 PREGNANTRIOL	196123	247.6
22.26 TETRAHYDROCORTICOL	527183	1385.6

Metil/ET	52.3
Limbol/ET	93.4
ET/ET	1.8
Limbol/ET	1.0
ET/ET	1.5
ET/ET	0.1
ET/ET	1.1
ET/ET	19.5
ET/ET	1.8

DATA: 7-040313 (1337) 18 D. SPMS 342.850  
 Muestra:  
 Posición: Landa 04127073A MONO 041220 VIAL 16

13 Sep 2004 20:31  
 METODO: 2MONOB.M



El 3 de monobusado es 10.37  
 Area/oc/5551=0.41

LA REACCIÓN metil alcohol: 0.87  
 LA REACCIÓN metil: 2.21  
 LA REACCIÓN etanol: 3.55

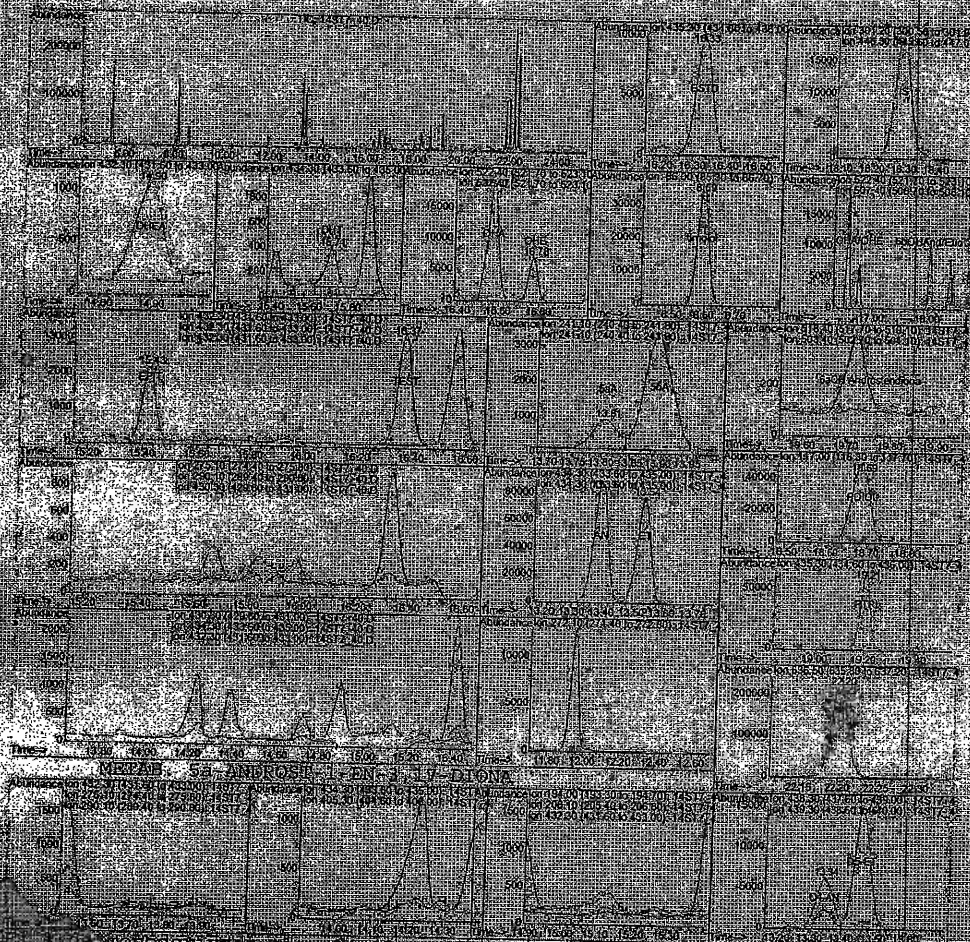
12.30 METILTESTOSTERONA	52732	250
3.60 ALCOHOL	95310	238
1.41 GLS ANDROSTERONA	449318	2605
17.52 PISCOLANOLONA	514861	2702
13.41 6-ADRIOL	3924	32.4
13.80 5-ADRIOL	10042	124.9
14.90 DHA	6003	27.4
15.44 BPTESTOSTERONA	10895	29.4
15.72 DBO	2379	17.6
16.37 TESTOSTERONA	18620	33.9
16.58 CHA	111859	698.8
16.78 OFE	22110	184.2
18.62 PREGNANTRIOL	294152	277.7
19.21 PREGNANTRIOL	241549	1425.1
22.71 ETETRAHROCORTISOL	699481	2378.0

[An]/[Et]	65.3
[An]/[Et+D]	88.5
[E]/[Et+D]	1.4
[An]/[Et+D]	1.0
[OEt]/[OEt]	3.18
[5ADRIOL]/[5ADRIOL]	0.2
[5ADRIOL]/[5ADRIOL]	1.1
[MDH]/[Et+D]	0.5
[DHT]/[DHT]	0.6



DATA W 040914 14ST7 40 B Sample: 348 368  
 Muestra:  
 Position: Banda: 04127091 AMONOD 041223 VIAL: B

14 Sep 2004 16:41  
 METODO: 7MONOD.M



11-13 ANDROSTADIEN-3-OL	32178	259		
11-13 ANDROSTADIEN-3-OL	32168	233		
11-13 ANDROSTADIEN-3-OL	284624	1816		
11-13 ANDROSTADIEN-3-OL	246017	1301		
11-13 ANDROSTADIEN-3-OL	2693	22.3	[And]/[T] =	55.4
11-13 ANDROSTADIEN-3-OL	10541	153.1	[And]/[Epi] =	85.1
11-13 ANDROSTADIEN-3-OL	2916	13.4		
11-13 ANDROSTADIEN-3-OL	7123	19.4	[T]/[Epi] =	1.3
11-13 ANDROSTADIEN-3-OL	1284	9.6	[And]/[Epi] =	1.1
11-13 ANDROSTADIEN-3-OL	11719	25.2	[OH]/[OH] =	2.2
11-13 ANDROSTADIEN-3-OL	74876	210.9		
11-13 ANDROSTADIEN-3-OL	23806	200.3	[5αADIOL]/[5αADIOL] =	0.1
11-13 ANDROSTADIEN-3-OL	153512	245.9	[5αADIOL]/[Epi] =	1.0
11-13 ANDROSTADIEN-3-OL	174945	1330.8	[MDH]/[Epi] =	7.4
11-13 ANDROSTADIEN-3-OL	373381	1276.1	[DHE]/[Epi] =	0.5

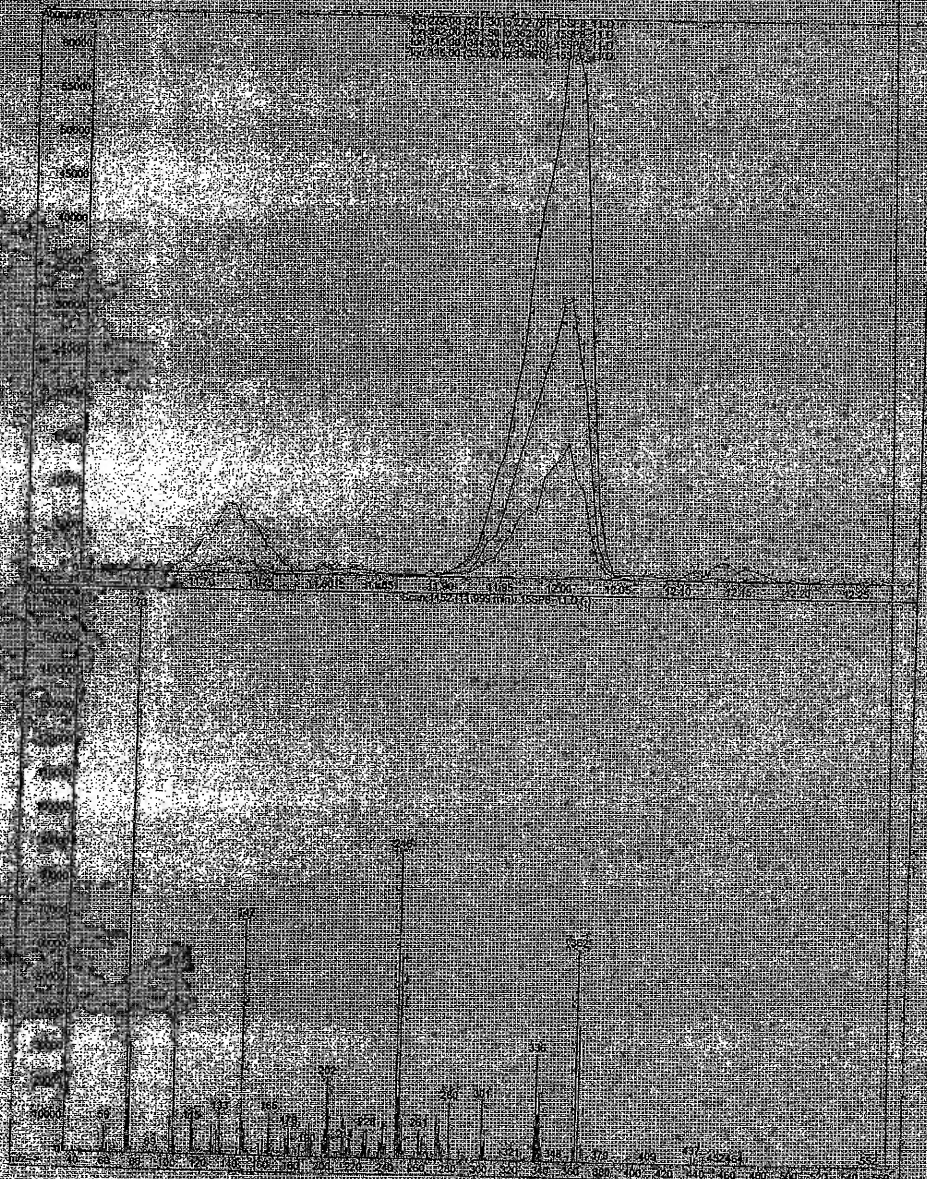
# VIA

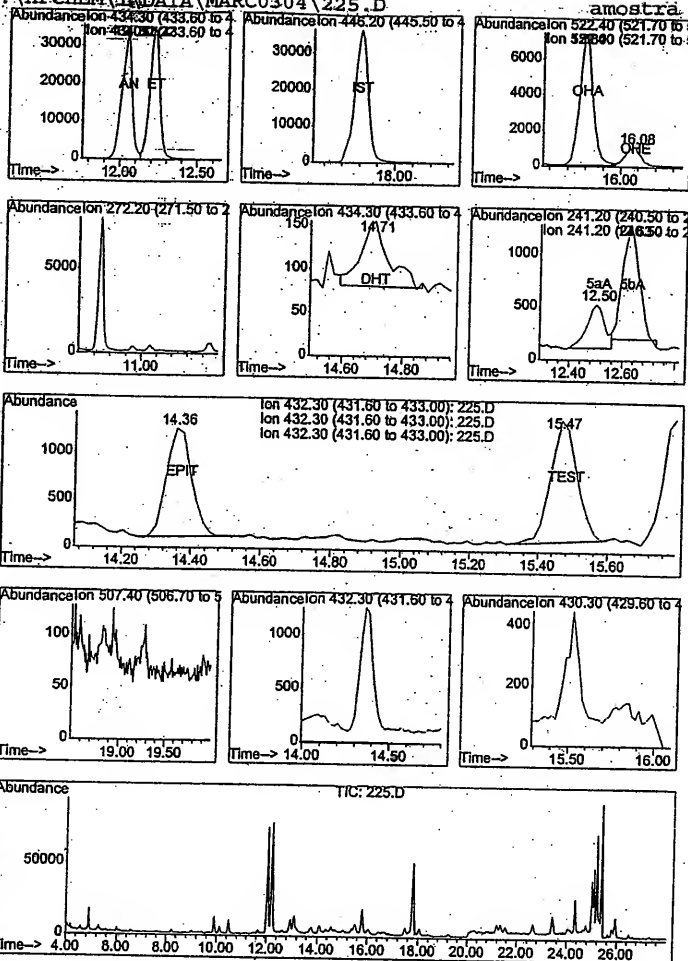
[An] / [T] =	68.9
[An] / [EpT] =	66.8
[T] / [BoT] =	1.0
[An] / [EpT] =	1.3
[OHA] / [OHE] =	2.2
[SaadTol] / [SaadTol] =	0.1
[SaadTol] / [EpT] =	0.9
[OHT] / [EpT] =	10.3
[OHT] / [BoT] =	0.6



File: D:\DATA\040915\15SP8\_11.D  
 Operator: MSG 15/09/04  
 Acquired: 15 Sep 2004 17:22 using AcqMethod: 806-043C  
 Instrument: EQ-03-08  
 Sample Name: 04127126A.SU 1219-11  
 Misc Info:  
 Vial Number: 8

INTERFERENCIA EN VENTANA DE MONITORING





RTE6.D 14 Oct 1999 4:48 pRTE 5.2

- factor de [E] en RTE6= 0.01971

- factor de [T] en RTE6= 0.10178

17.81 Metiltestosterona	1955845	500	[T] / [EpiT] =	1.3
12.05 Androsterona	1494348	488	[T] corr =	20.2
12.23 Etiocolanolona	1564327	415.0	[E] corr =	15.0
14.37 Epitestosterona	57829	10.4	[An] / [T] =	45.2
15.48 Testosterona	67141	10.8	[An] / [EpiT] =	47.0
15.81 11 OH Androsterona	382508	146.8		
16.08 11 OH Etiocolanolona	191956	124.9	[T] / [EpiT] =	1.0
12.51 5 a Diol	17942	10.4	[An] / [Eti] =	1.2
12.64 5 b Diol	47406	21.5	[OHA] / [OHE] =	1.2
14.71 DHT	4747	3.6		
			[5aADIOL] / [5bADIOL] =	0.5
			[5aADIOL] / [EpiT] =	1.0
			[mDHT] / [Eti] =	8.8
			[DHT] / [EpiT] =	0.4

V=  
 XIMO= 0.0  
 MINIMO= 0.0

# **Laboratório de Análises e Dopagem**

Cadeia de Custódia - Folha de Recepção

Rel. Nº RELT-LADB-D- 069/04

Código: GENIAL

MOD-LADB-060 REV:00

Nº Lab	Frasco A nº	Composição	Modelidade	Sexo	pH (local)	Data (local)	Vol. (ml) (local)	Medicamentos Declarados	Observações
0224	276128	C.I.	CICLISMO	M	5	1019	90	POLIVITAMINAS	caprina
0225	277821	C.I.	CICLISMO	M	6	1013	120	SEREVENT	caprina
0226	277822	C.I.	CICLISMO	M	5	1020	90		caprina
0227	277824	C.I.	CICLISMO	M	6	1025	110		a esta velocidade muito zolamida

Resp. Cadeia de Custódia

Data de Entrada no LADB

Director Científico

Julia  
25/02/04

25  
25/02/04

21  
21/04/04



3873 GRAND VIEW BLVD  
LOS ANGELES, CA 90066  
PHONE 310-482-6925  
FAX 310-482-6929

---

**FACSIMILE TRANSMITTAL SHEET**

---

TO:	FROM:
Travis Tygart	Don H. Catlin, M.D.
COMPANY:	DATE:
USADA	3/30/2007
FAX NUMBER:	TOTAL NO. OF PAGES INCLUDING COVER:
(719) 785-2001	41
PHONE NUMBER:	SENDER'S REFERENCE NUMBER:
RE:	YOUR REFERENCE NUMBER:

---

☒ URGENT    ☐ FOR REVIEW    ☐ PLEASE COMMENT    ☐ PLEASE REPLY    ☐ PLEASE RECYCLE

---

**CONFIDENTIAL**

Requested profiles.



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

466193  
12/8/02

Sample Name : YWI  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 13 Dec 2002 7:21 am  
Method File : ANAB001

Data File: YWI08.D  
Equipment # : MSDA6  
ALS Bottle # : 30

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.425	1.000	43779 *	<40.0>
ANDROSTERONE--434	10.734	0.800	447168	1815.5
ETIOCHOLANOLONE--434	10.936	0.815	462784	1810.7
Mono-Androsterone--272	9.294	0.692	1758	
TESTOSTERONE--432	13.469	1.003	17309	16.5
EPITESTOSTERONE--432	12.607	0.939	15379	16.0
DHT-DIHYDROTESTOSTERONE--434	12.822	0.955	699	2.4
5a-Androstan-3a,17B-diol--241	11.073	0.825	10910	22.9
5B-Androstan-3a,17B-diol--241	11.229	0.836	33523	78.3
11-OH-ANDROST--522	13.815	1.029	65232	271.6
11-OH-ETIOCHO--522	14.097	1.050	89888	456.7
DHEA--432	12.083	0.900	9086	
VIT E METABOLITE--422	9.173	0.683	32031	
CORTISOL METAB --462	17.474	1.302	23309	

\* Height shown is d3T - 1% d0-T Peakheight.

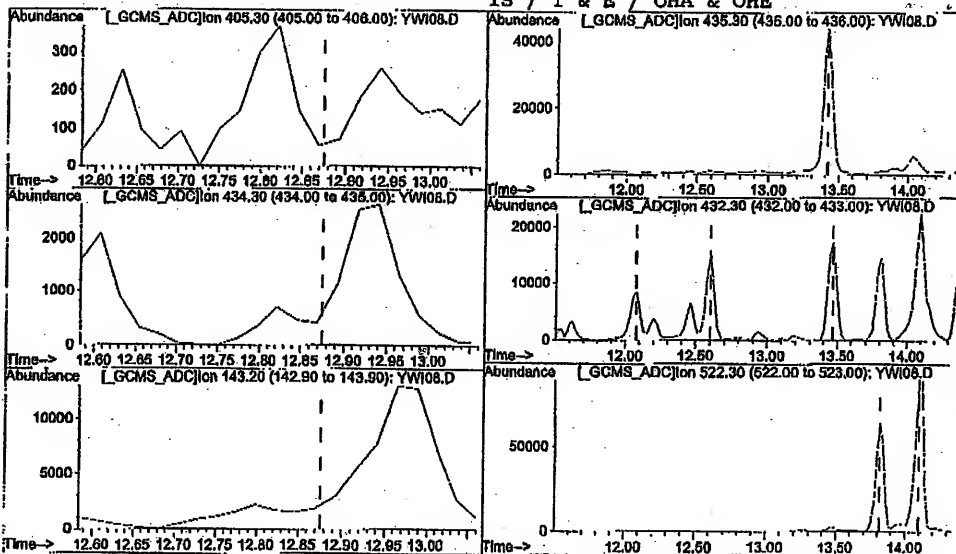
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8612
EIS 462-D3T	3.9100-4.1100	4.0490
D3T-VIT E MET	4.1600-4.3600	4.2525

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

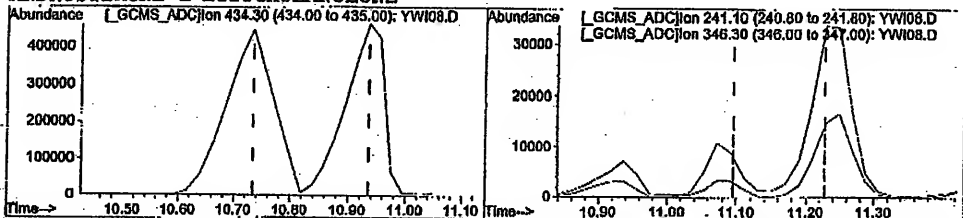
TESTOSTERONE	/	EPITESTOSTERONE (4)	1.13
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	1.0
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	0.7
ANDROSTERONE	/	EPITESTOSTERONE	29.1
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	0.4 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	1.4
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 10.8
D0-Testosterone	/	D3-Testosterone	0.4

## Analysis Report for Data File = YWI08.D Graphics Page 1

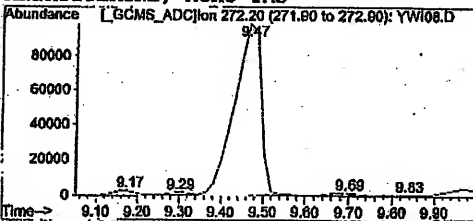
IS / T &amp; B / OHA &amp; OHE



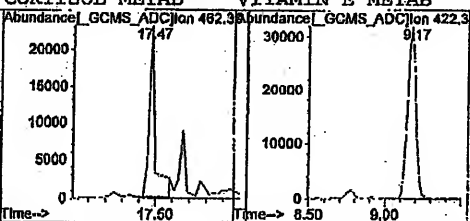
## ANDROSTERONE &amp; ETIOCHOLANOLONE



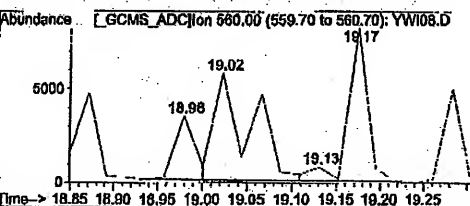
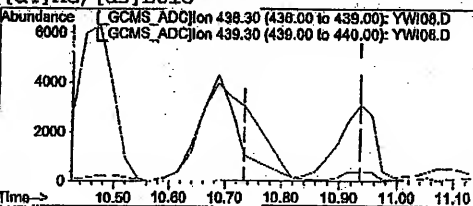
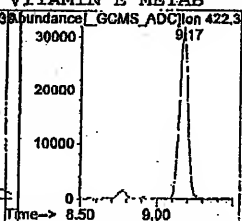
## ANDROSTERONE, Mono-TMS



## CORTISOL METAB



## VITAMIN E METAB



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

476315  
11/26/03

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 1ER  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 4 Dec 2003 4:45 am  
Method File : ANAB002

Data File: 1ER03.D  
Equipment # : msda7  
ALS Bottle # : 19

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.492	1.000	39414 *	<40.0>
ANDROSTERONE--434	10.819	0.802	240768	859.3
ETIOCHOLANOLONE--434	11.014	0.816	523648	1632.8
Mono-Androsterone--272	9.365	0.694	3023	
TESTOSTERONE--432	13.535	1.003	17763	14.0
EPITESTOSTERONE--432	12.673	0.939	16155	88.1
DHT=DIHYDROTESTOSTERONE--434	12.897	0.956	993	3.8
5a-Androstan-3a,17B-diol--241	11.151	0.827	7893	37.3
5B-Androstan-3a,17B-diol--241	11.307	0.838	27438	153.2
11-OH-ANDROST--522	13.903	1.031	116824	409.8
11-OH-ETIOCHO--522	14.185	1.051	124136	460.1
DHEA--432	12.150	0.901	8116	
VIT E METABOLITE--422	9.243	0.685	164260	
CORTISOL METAB --462	17.585	1.303	64121	

\* Height shown is d3T - 1% d0-T Peakheight.

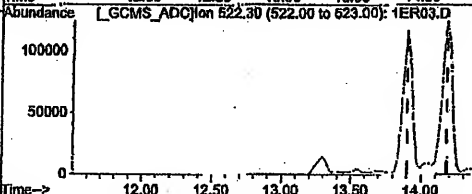
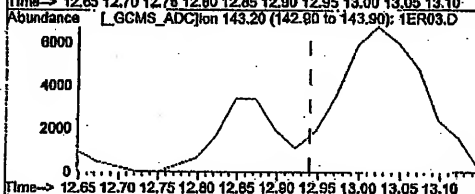
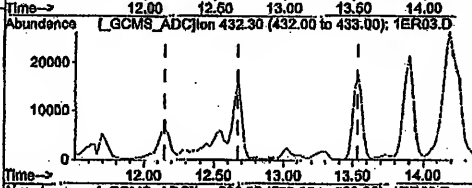
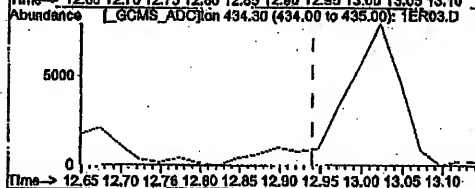
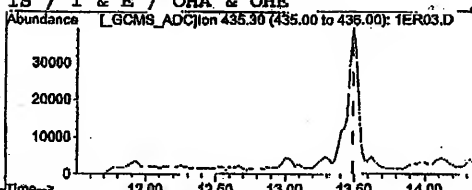
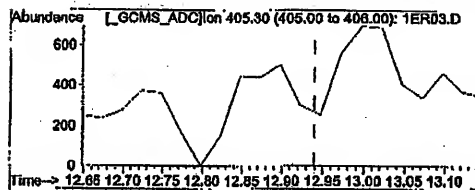
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8618
EIS 462-D3T	3.9100-4.1100	4.0932
D3T-VIT E MET	4.1600-4.3600	4.2487

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

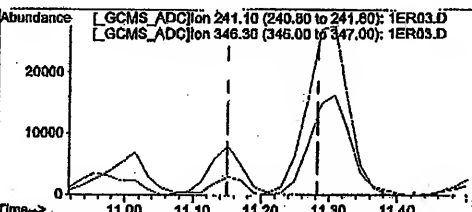
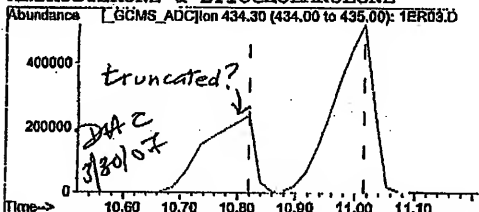
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.10
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.5
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	0.9
ANDROSTERONE	/ EPITESTOSTERONE	14.9
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	1.3 %
DHT	/ EPITESTOSTERONE (1.8)	0.0
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	0.4
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 8.1
D0-Testosterone	/ D3-Testosterone	0.5

## Analysis Report for Data File = 1ER03.D Graphics Page 1

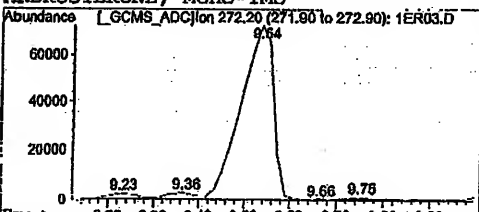
IS / T &amp; E / OHA &amp; OHE



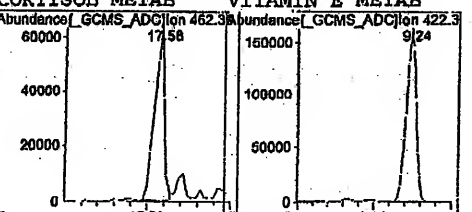
## ANDROSTERONE &amp; ETIOCHOLANOLONE



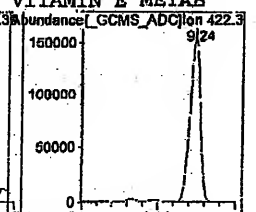
## ANDROSTERONE, Mono-TMS



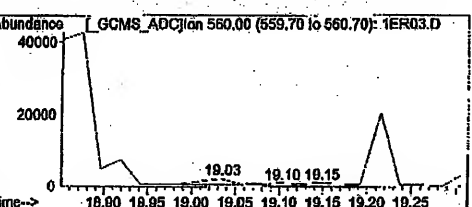
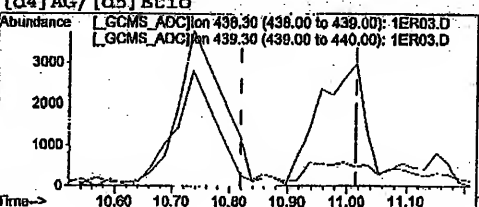
## CORTISOL METAB



## VITAMIN E METAB



## [d4] AG / [d5] Bt1c



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

&gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 4JF  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 10 Jan 2005 10:20 pm  
Method File : ANAB004

Data File: 4JF04.D

Equipment # : MSDA8  
ALS Bottle # : 33

485155  
1/8/05

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.462	1.000	44112 *	<40.0>
ANDROSTERONE--434	10.830	0.805	219776	932.7
ETIOCHOLANOLONE--434	11.005	0.818	351168	1310.4
Mono-Androsterone--272	9.321	0.692	575	
TESTOSTERONE--432	13.505	1.003	17646	17.2
EPITESTOSTERONE--432	12.676	0.942	21942	23.8
DHT=DIHYDROTESTOSTERONE--434	12.883	0.957	1080	4.6
5a-Androstan-3a,17B-diol--241	11.162	0.829	11209	39.4
5B-Androstan-3a,17B-diol--241	11.279	0.838	30202	98.2
11-OH-ANDROST--522	13.852	1.029	28216	87.5
11-OH-ETIOCHO--522	14.112	1.048	6198	23.5
DHEA--432	12.156	0.903	6906	
VIT E METABOLITE--422	9.197	0.683	95910	
CORTISOL METAB --462	17.676	1.313	8359	

\* Height shown is d3T - 1% d0-T Peakheight.

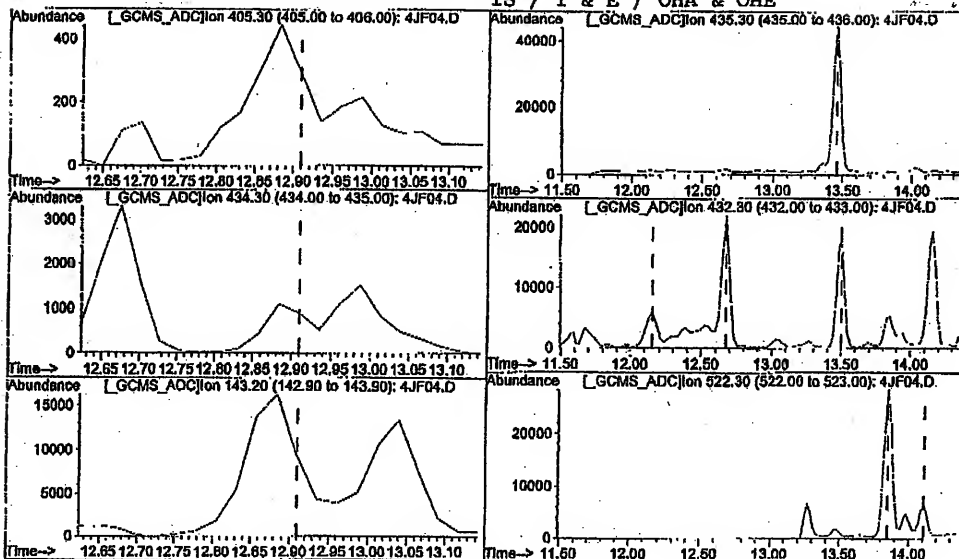
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8294
EIS 462-D3T	3.9100-4.1100	4.2141
D3T-VIT E MET	4.1600-4.3600	4.2649

&gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

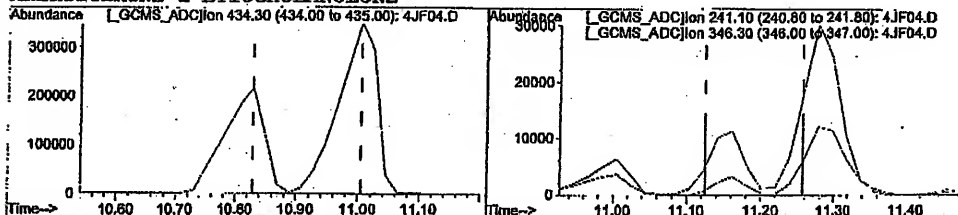
TESTOSTERONE	/ EPITESTOSTERONE (4)	0.80
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.6
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	4.6
ANDROSTERONE	/ EPITESTOSTERONE	10.0
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	0.3 %
DHT	/ EPITESTOSTERONE (1.0)	0.2
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.7
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.4
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 2.8
D0-Testosterone	/ D3-Testosterone	0.4

## Analysis Report for Data File = 4JF04.D Graphics Page 1

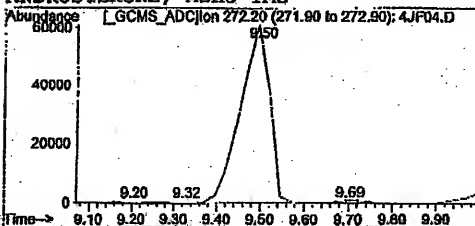
IS / T &amp; E / OHA &amp; OHE



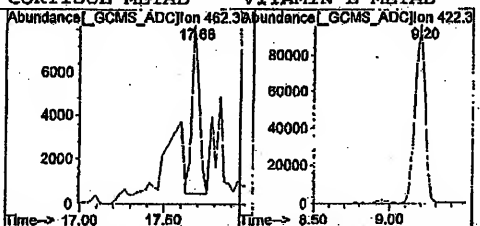
## ANDROSTERONE &amp; ETIOCHOLANOLONE



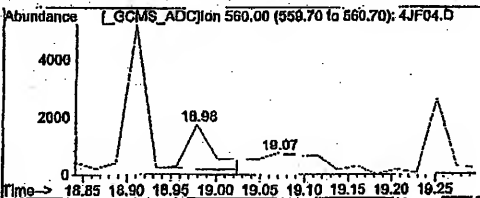
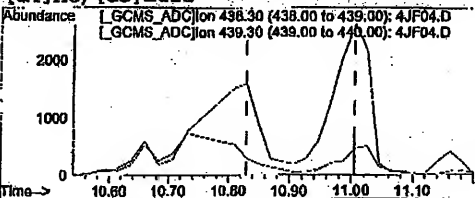
## ANDROSTERONE, Mono-TMS



## CORTISOL METAB VITAMIN E METAB



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

920460  
4/21/05

&gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 5FP  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 25 Apr 2005 2:50 am  
Method File : ANAB004

Data File: 5FP12.D  
Equipment # : MSDA12  
ALS Bottle # : 7

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.418	1.000	37020 *	<40.0>
ANDROSTERONE--434	10.715	0.799	151040	709.7
ETIOCHOLANOLONE--434	10.898	0.812	140864	697.0
Mono-Androsterone--272	9.292	0.693	620	
TESTOSTERONE--432	13.461	1.003	9228	10.5
EPITESTOSTERONE--432	12.608	0.940	6053	7.4
DHT=DIHYDROTESTOSTERONE--434	12.813	0.955	254	1.0
5a-Androstan-3a,17B-diol--241	11.078	0.826	3633	8.7
5B-Androstan-3a,17B-diol--241	11.213	0.836	11197	31.8
11-OH-ANDROST--522	13.847	1.032	62488	297.7
11-OH-ETIOCHO--522	14.082	1.050	16744	99.8
DHEA--432	12.068	0.899	5698	
VIT E METABOLITE--422	9.185	0.685	215798	
CORTISOL METAB --462	17.456	1.301	6462	

\* Height shown is d3T - 1% d0-T Peakheight.

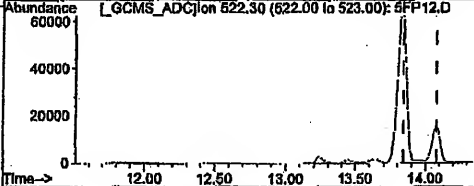
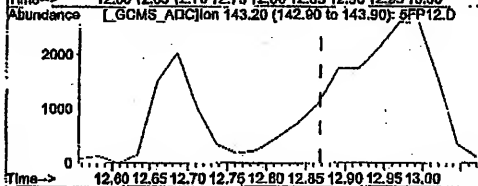
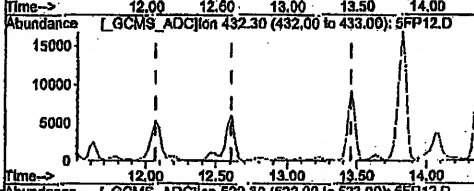
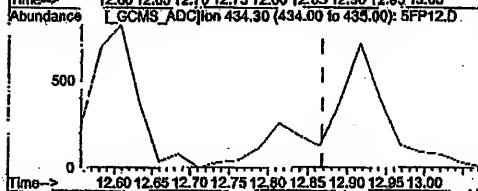
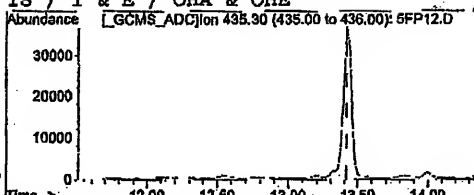
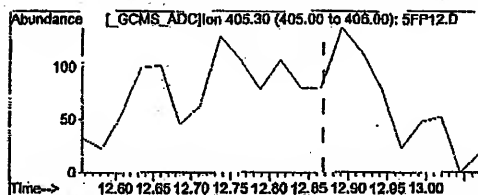
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8529
EIS 462-D3T	3.9100-4.1100	4.0379
D3T-VIT E MET	4.1600-4.3600	4.2330

&gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

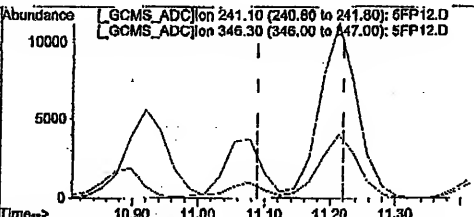
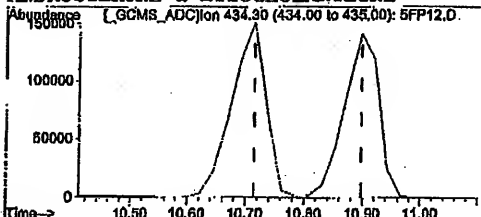
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.52
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	1.1
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	3.7
ANDROSTERONE	/ EPITESTOSTERONE	25.0
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	0.4 %
DHT	/ EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.2
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 11.5
D0-Testosterone	/ D3-Testosterone	0.2

## Analysis Report for Data File 5FP12.D Graphics Page 1

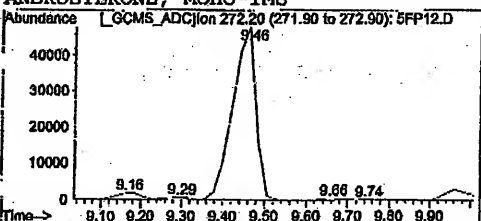
IS / T &amp; E / OHA &amp; OHE



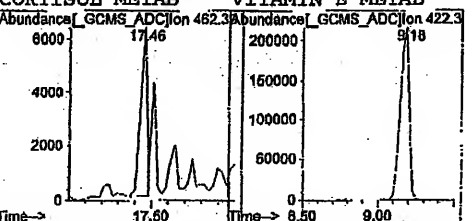
## ANDROSTERONE &amp; ETIOCHOLANOLONE



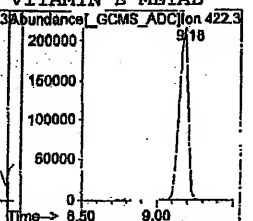
## ANDROSTERONE, Mono-TMS



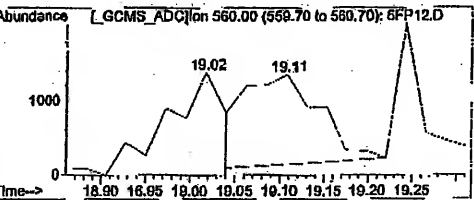
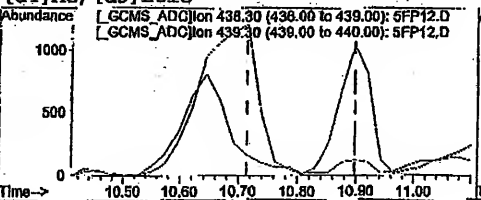
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]E1o





PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

920462  
4/22/05

Sample Name : SHF  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 28 Apr 2005 8:10 pm  
Method File : ANABO04

Data File: SHF05.D  
Equipment # : MSDA12  
ALS Bottle # : 13

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T---435	13.482	1.000	29746 *	<40.0>
ANDROSTERONE--434	10.785	0.800	236928	1415.7
ETIOCHOLANOLONE--434	10.988	0.815	258944	1491.1
Mono-Androsterone--272	9.356	0.694	1209	
TESTOSTERONE--432	13.504	1.002	17414	24.4
EPITESTOSTERONE--432	12.634	0.937	13999	21.4
DHT=DIHYDROTESTOSTERONE--434	12.865	0.954	445	2.2
5a-Androstan-3a,17b-diol--241	11.123	0.825	9718	30.0
5b-Androstan-3a,17b-diol--241	11.258	0.835	31927	109.8
11-OH-ANDROST--522	13.890	1.030	93904	575.4
11-OH-ETIOCHO--522	14.147	1.049	39488	295.3
DHEA--432	12.145	0.901	8440	
VIT E METABOLITE--422	9.249	0.686	388810	
CORTISOL METAB --462	17.456	1.295	19108	

\* Height shown is d3T - 1% d0-T Peakheight.

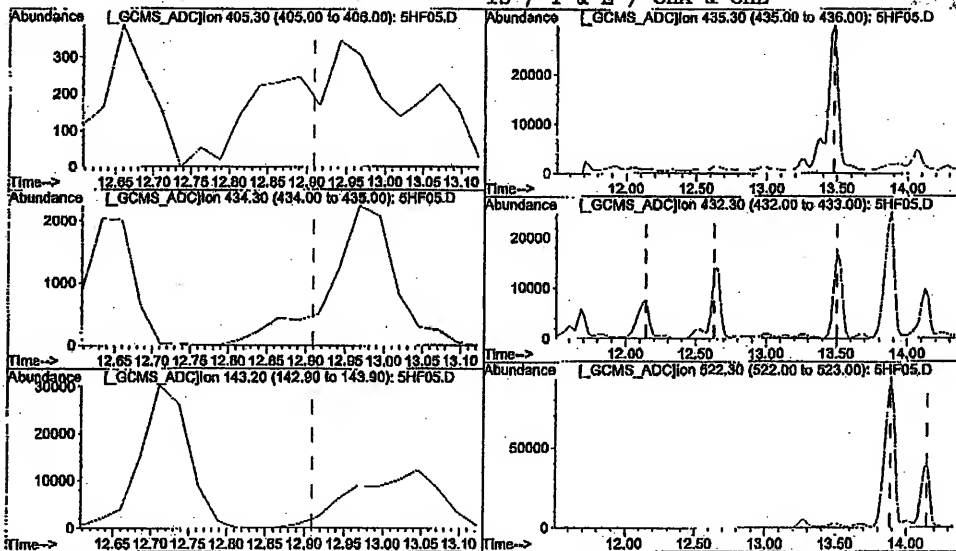
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8701
EIS 462-D3T	3.9100-4.1100	3.9736
D3T-VIT E MET	4.1600-4.3600	4.2330

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

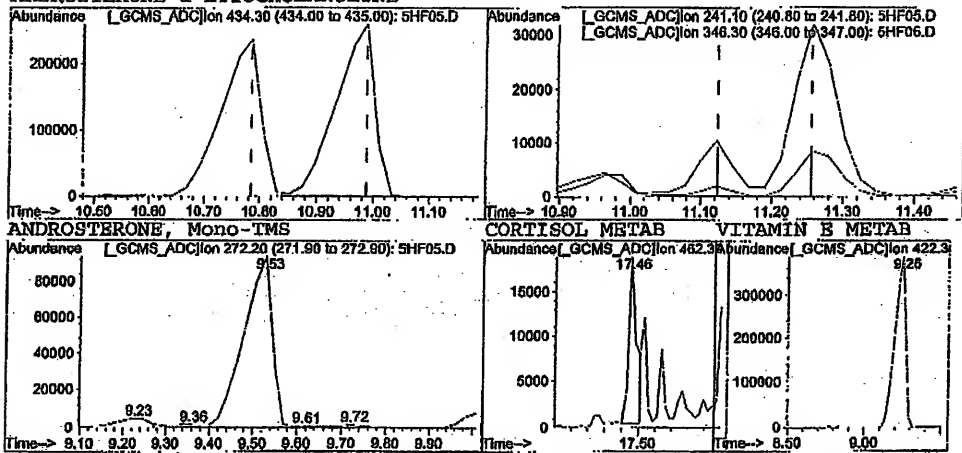
TESTOSTERONE	/	EPITESTOSTERONE (4)	1.24
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	0.9
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	2.4
ANDROSTERONE	/	EPITESTOSTERONE	16.9
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	0.5 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	1.4
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 5.8
D0-Testosterone	/	D3-Testosterone	0.6

## Analysis Report for Data File - 5HF05.D Graphics Page 1

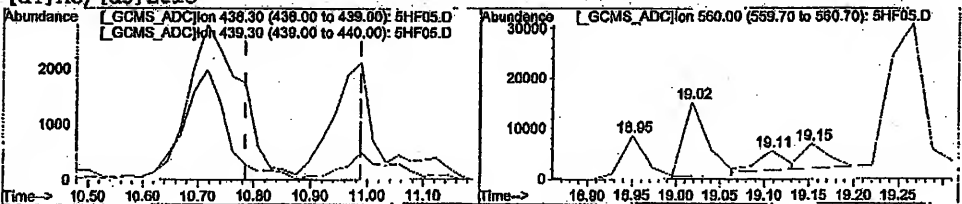
IS / T &amp; E / OHA &amp; OHE



## ANDROSTERONE &amp; ETIOCHOLANOLONE



## [d4]AG / [d5] Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

491607  
10/7/05

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 63L  
Miscellaneous: WU:A UCAL 2.5 mL NH4I  
Analysis Time: 12 Oct 2005 7:24 pm  
Method File : ANABO04

Data File: 63L03.D  
Equipment # : MSDA15  
ALS Bottle # : 7

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.493	1.000	28617 *	<40.0>
ANDROSTERONE--434	10.908	0.808	467776	1898.0
ETIOCHOLANOLONE--434	11.132	0.825	713984	2458.8
Mono-Androsterone--272	9.338	0.692	5852	
TESTOSTERONE--432	13.536	1.003	29522	40.6
EPITESTOSTERONE--432	12.700	0.941	27122	41.9
DHT=DIHYDROTESTOSTERONE--434	12.906	0.956	1964	11.0
5a-Androstan-3a,17B-diol--241	11.255	0.834	42644	138.2
5B-Androstan-3a,17B-diol--241	11.377	0.843	137432	434.7
11-OH-ANDROST--522	13.879	1.029	76232	521.4
11-OH-ETIOCHO--522	14.115	1.046	7665	65.2
DHEA--432	12.186	0.903	5859	
VIT E METABOLITE--422	9.231	0.684	525189	
CORTISOL METAB --462	17.595	1.304	42953	

\* Height shown is d3T - 1% d0-T Peakheight.

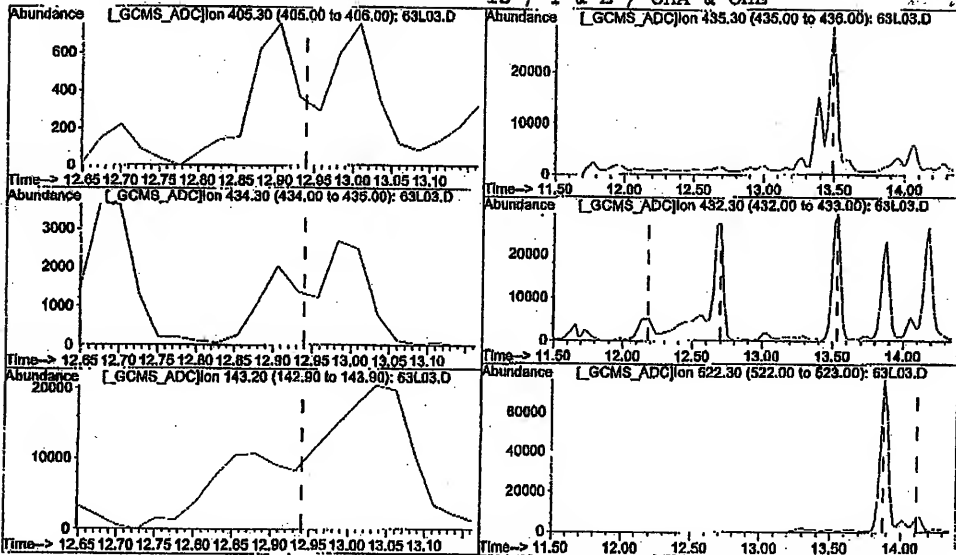
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8358
EIS 462-D3T	3.9100-4.1100	4.1015
D3T-VIT E MET	4.1600-4.3600	4.2624

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

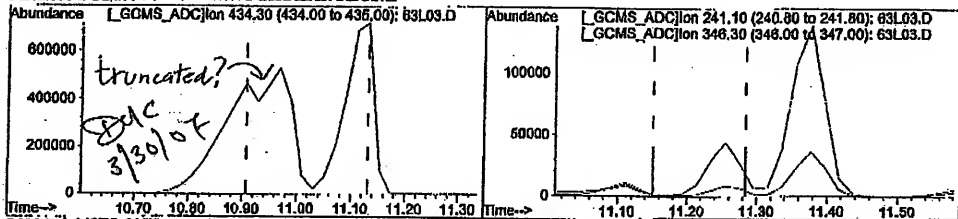
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.09
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.7
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	9.9
ANDROSTERONE	/ EPITESTOSTERONE	17.2
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	1.3 %
DHT	/ EPITESTOSTERONE (1.8)	0.3
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	3.3
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	0.9
D0-Testosterone	/ D3-Testosterone	1.0

## Analysis Report for Data File = 63L03.D Graphics Page 1

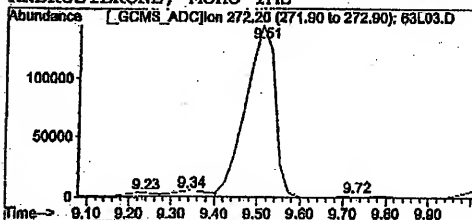
IS / T &amp; E / OHA &amp; OHE



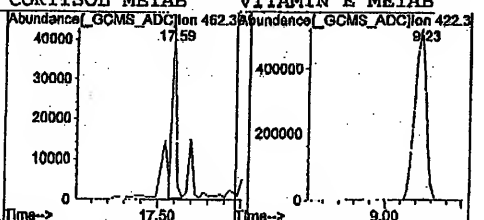
## ANDROSTERONE &amp; ETIOCHOLANOLONE



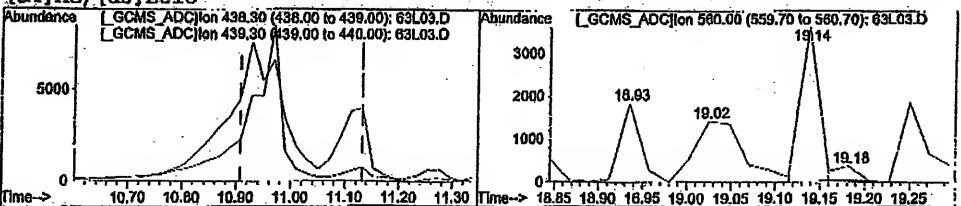
## ANDROSTERONE, Mono-TMS



## CORTISOL METAB



## [d4] AG / [d5] Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

493084  
11/5/05

Sample Name : 6W9  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 18 Nov 2005 3:19 pm  
Method File : ANAB004

Data File: 6W903.D  
Equipment # : msda4  
ALS Bottle # : 33

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.493	1.000	38678 *	<40.0>
ANDROSTERONE--434	10.904	0.808	191616	1367.4
ETIOCHOLANOLONE--434	11.068	0.820	263616	1668.0
Mono-Androsterone--272	9.338	0.692	371	
TESTOSTERONE--432	13.536	1.003	21790	29.8
EPITESTOSTERONE--432	12.689	0.940	20086	30.0
DHT-DIHYDROTESTOSTERONE--434	12.897	0.956	739	2.7
5a-Androstan-3a,17B-diol--241	11.213	0.831	6545	75.0
5B-Androstan-3a,17B-diol--241	11.336	0.840	28510	255.1
11-OH-ANDROST--522	13.883	1.029	60712	1643.1
11-OH-ETIOCHO--522	14.143	1.048	32272	1260.4
DHEA--432	12.170	0.902	6970	
VIT E METABOLITE--422	9.256	0.686	308200	
CORTISOL METAB --462	17.616	1.306	18294	

\* Height shown is d3T - 1% d0-T Peakheight.

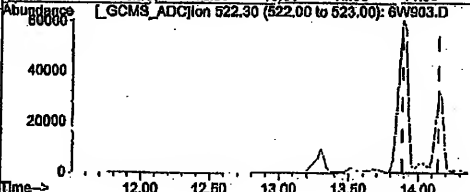
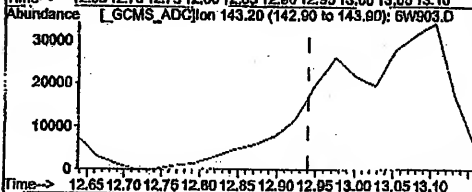
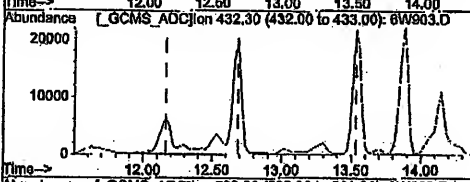
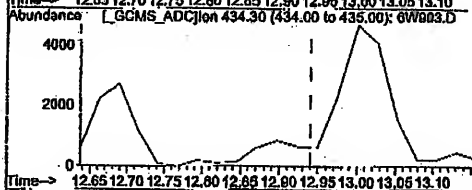
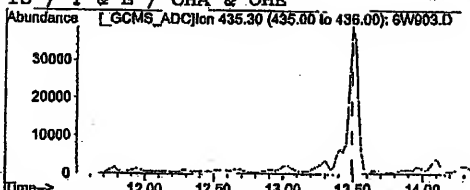
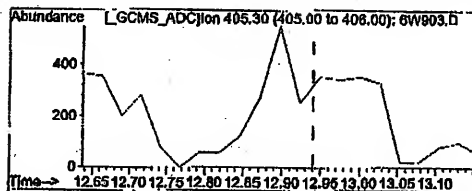
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8469
EIS 462-D3T	3.9100-4.1100	4.1231
D3T-VIT E MET	4.1600-4.3600	4.2367

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

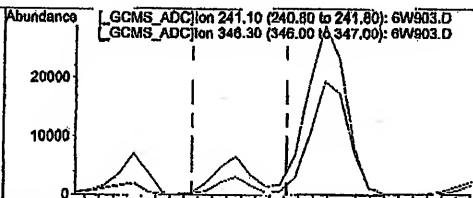
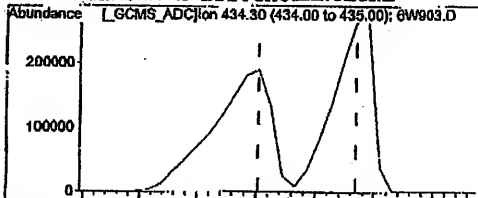
TESTOSTERONE	/	EPITESTOSTERONE (4)	1.08
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	0.7
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	1.9
ANDROSTERONE	/	EPITESTOSTERONE	9.5
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	0.2 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	2.5
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 22.9
D0-Testosterone	/	D3-Testosterone	0.6

## Analysis Report for Data File = 6W903.D Graphics Page 1

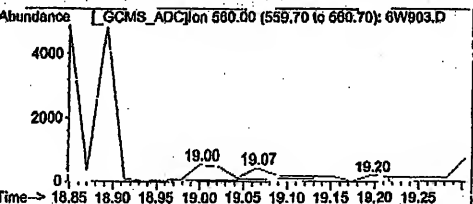
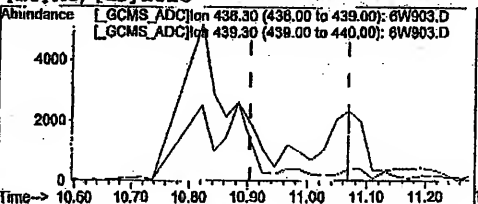
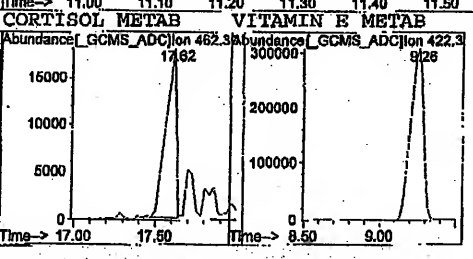
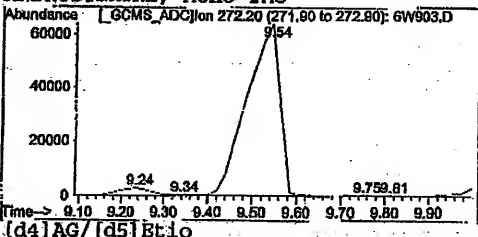
IS / T &amp; E / OHA &amp; OHE



## ANDROSTERONE &amp; ETIOCHOLANOLONE



## ANDROSTERONE, Mono-TMS



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

493091  
1/6/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 7YG  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 13 Jan 2006 3:45 am  
Method File : ANABO04

Data File: 7YG09.D  
Equipment # : MSDA13  
ALS Bottle # : 28

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.493	1.000	4281 *	<40.0>
ANDROSTERONE--434	10.786	0.799	23912	1283.8
ETIOCHOLANOLONE--434	11.030	0.817	70696	3984.5
Mono-Androsterone--272	9.231	0.684	578	
TESTOSTERONE--432	13.536	1.003	3083	29.5
EPITESTOSTERONE--432	12.674	0.939	3446	33.7
DHT-DIHYDROTESTOSTERONE--434	12.880	0.955	122	3.8
5a-Androstan-3a,17B-diol--241	11.173	0.828	907	40.1
5B-Androstan-3a,17B-diol--241	11.295	0.837	5852	252.9
11-OH-ANDROST--522	13.879	1.029	5768	862.9
11-OH-ETIOCHO--522	14.136	1.048	6894	1328.4
DHEA--432	12.160	0.901	1181	
VIT E METABOLITE--422	9.231	0.684	40326	
CORTISOL METAB --462	17.517	1.298	763	

\* Height shown is d3T - 1% d0-T Peakheight.

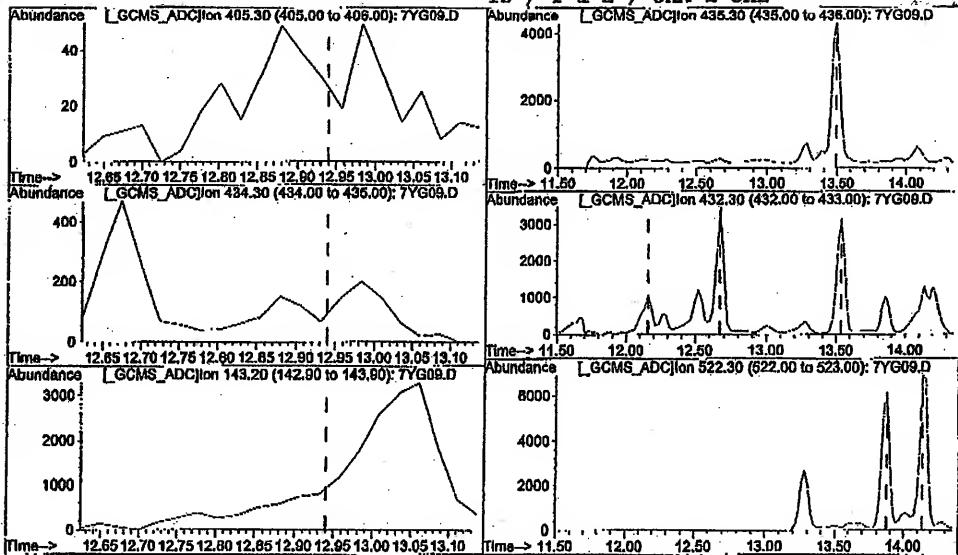
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8615
EIS 462-D3T	3.9100-4.1100	4.0244
D3T-VIT E MET	4.1600-4.3600	4.2624

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

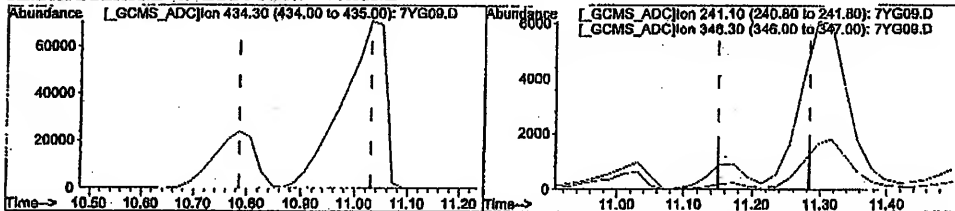
TESTOSTERONE	/	EPITESTOSTERONE (4)	0.89
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	0.3
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	0.8
ANDROSTERONE	/	EPITESTOSTERONE	6.9
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	2.4 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	1.2
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL(3)	0.2
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 17.7
D0-Testosterone	/	D3-Testosterone	0.7

## Analysis Report for Data File = 7YG09.D Graphics Page 1

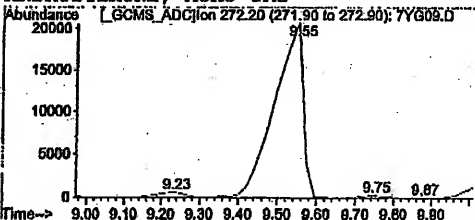
IS / T &amp; E / OHA &amp; OHE



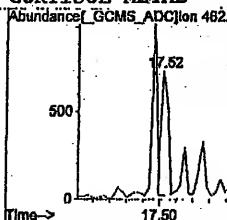
## ANDROSTERONE &amp; ETIOCHOLANOLONE



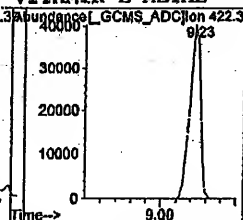
## ANDROSTERONE, Mono-TMS



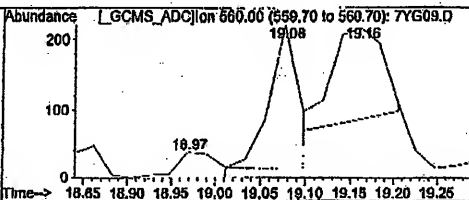
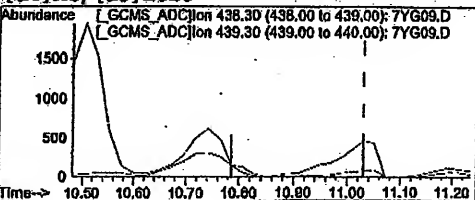
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Et10





PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

95/826  
2/22/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 7Y3  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 1 Mar 2006 3:57 pm  
Method File : ANAB004

Data File: 7Y303.D

Equipment # : MSDA13  
ALS Bottle # : 3

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.450	1.000	30109 *	<40.0>
ANDROSTERONE--434	10.766	0.800	185152	1006.1
ETIOCHOLANOLONE--434	10.970	0.816	260416	1148.3
Mono-Androsterone--272	9.381	0.697	1814	
TESTOSTERONE--432	13.493	1.003	21120	27.5
EPITESTOSTERONE--432	12.623	0.939	11960	16.2
DHT-DIHYDROTESTOSTERONE--434	12.855	0.956	412	1.7
5a-Androstan-3a,17B-diol--241	11.112	0.826	3989	17.5
5B-Androstan-3a,17B-diol--241	11.234	0.835	18756	85.7
11-OH-ANDROST--522	13.836	1.029	77272	2851.6
11-OH-ETIOCHO--522	14.115	1.049	55304	2589.2
DHEA--432	12.109	0.900	11608	
VIT E METABOLITE--422	9.274	0.689	474871	
CORTISOL METAB --462	17.437	1.296	12257	

\* Height shown is d3T - 1% d0-T Peakheight.

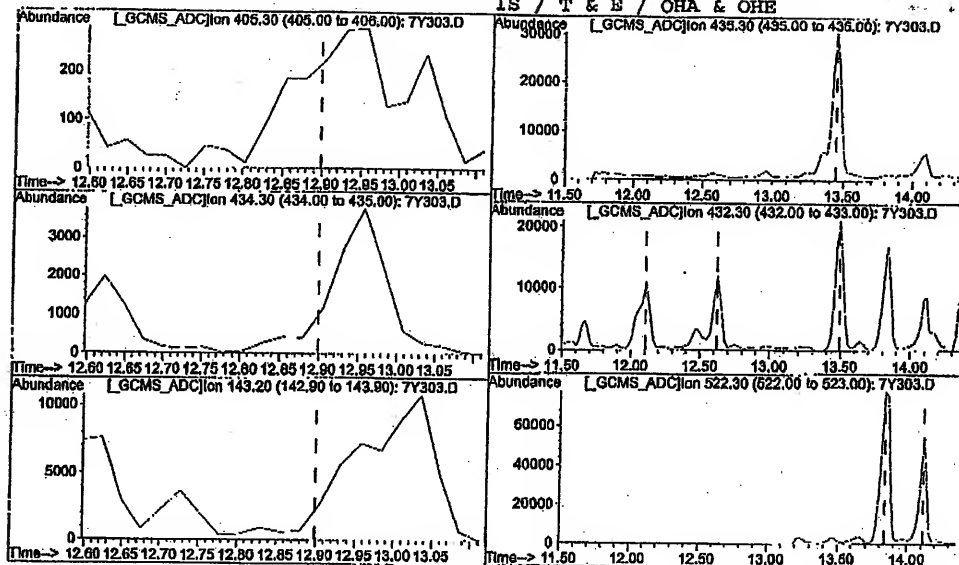
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8701
EIS 462-D3T	3.9100-4.1100	3.9869
D3T-VIT E MET	4.1600-4.3600	4.1766

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

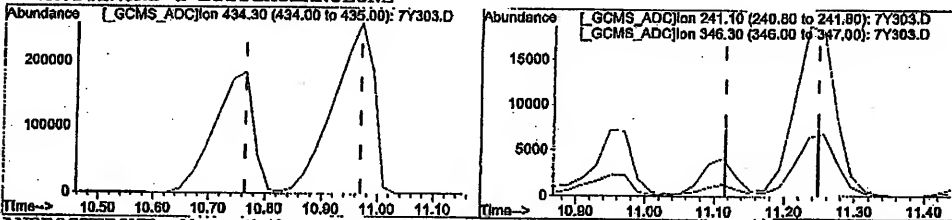
TESTOSTERONE	/	EPITESTOSTERONE (4)	1.77
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	0.7
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	1.4
ANDROSTERONE	/	EPITESTOSTERONE	15.5
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	1.0 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	1.1
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 4.5
D0-Testosterone	/	D3-Testosterone	0.7

## Analysis Report for Data File = 7Y303.D Graphics Page 1

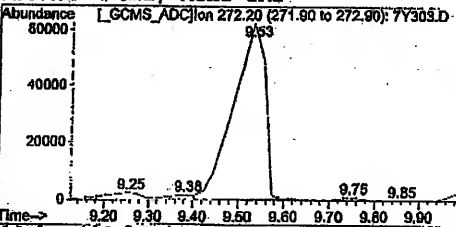
IS / T &amp; E / OHA &amp; OHE



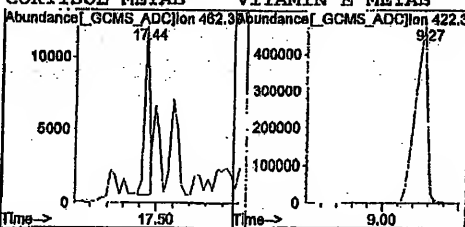
## ANDROSTERONE &amp; ETIOCHOLANOLONE



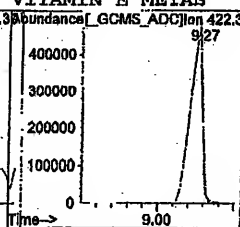
## ANDROSTERONE, Mono-TMS



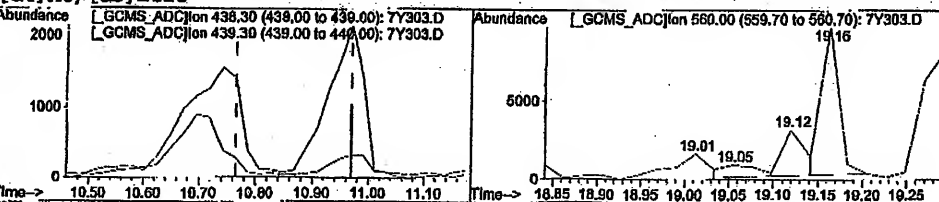
## CORTISOL METAB



## VITAMIN E METAB



## [d4] AG / [d5] Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

951875  
2/23/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 7Y3  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 1 Mar 2006 6:28 pm  
Method File : ANAB004

Data File: 7Y309.D  
Equipment # : MSDA13  
ALS Bottle # : 9

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.472	1.000	30426 *	<40.0>
ANDROSTERONE--434	10.786	0.801	227200	1221.7
ETIOCHOLANOLONE--434	11.010	0.817	293952	1282.6
Mono-Androsterone--272	9.252	0.687	2404	
TESTOSTERONE--432	13.515	1.003	22151	28.5
EPITESTOSTERONE--432	12.649	0.939	14423	19.3
DHT-DIHYDROTESTOSTERONE--434	12.983	0.964	1928	7.6
5a-Androstan-3a,17B-diol--241	11.133	0.826	4612	20.0
5B-Androstan-3a,17B-diol--241	11.275	0.837	15758	71.3
11-OH-ANDROST--522	13.879	1.030	101272	3698.3
11-OH-ETIOCHO--522	14.115	1.048	34984	1620.8
DHEA--432	12.135	0.901	7697	
VIT E METABOLITE--422	9.274	0.688	412005	
CORTISOL METAB --462	17.437	1.294	13408	

\* Height shown is d3T - 1% d0-T Peakheight.

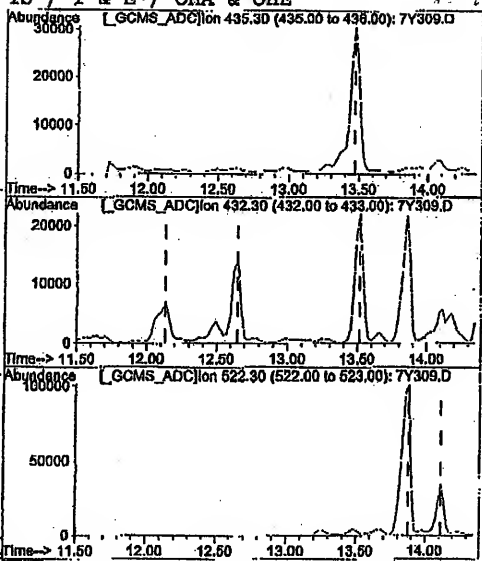
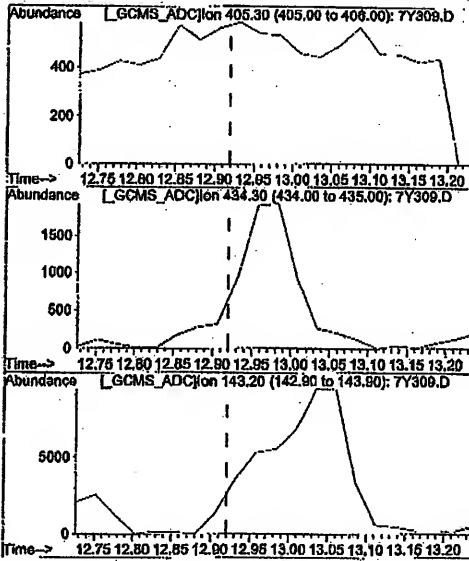
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8658
EIS 462-D3T	3.9100-4.1100	3.9655
D3T-VIT E MET	4.1600-4.3600	4.1981

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

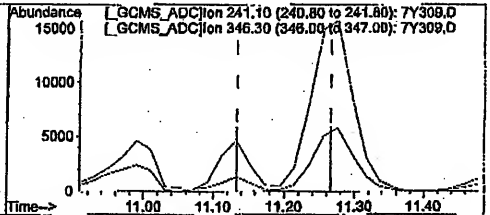
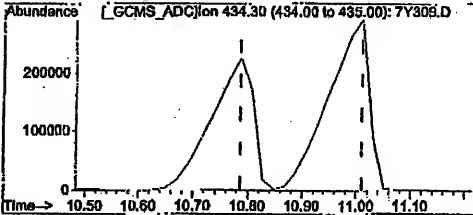
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.54
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.8
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	2.9
ANDROSTERONE	/ EPITESTOSTERONE	15.8
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	1.1 %
DHT	/ EPITESTOSTERONE (1.8)	0.4
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.0
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 4.2
D0-Testosterone	/ D3-Testosterone	0.7

## Analysis Report for Data File = 7Y309.D Graphics Page 1

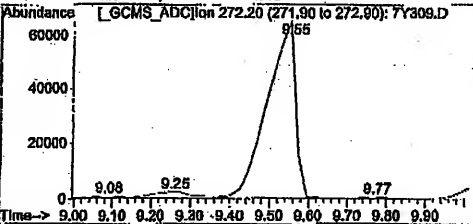
IS / T &amp; E / OHA &amp; OHE



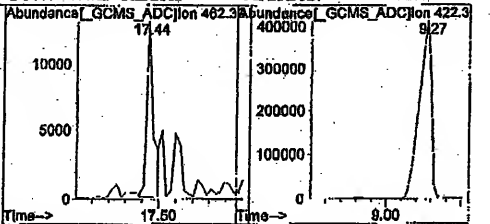
## ANDROSTERONE &amp; ETIOCHOLANOLONE



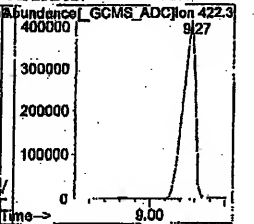
## ANDROSTERONE, Mono-TMS



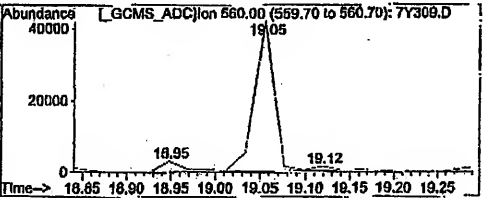
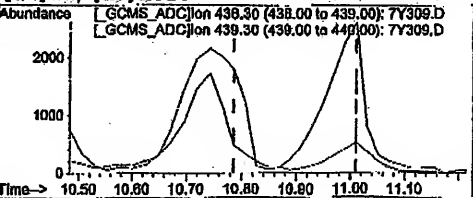
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

951828  
2/24/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 7Y9 B/W 7Y9  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 2 Mar 2006 11:41 pm  
Method File : ANABO04

Data File: 7Z604.D  
Equipment # : MSDA8  
ALS Bottle # : 37

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.482	1.000	10304 *	<40.0>
ANDROSTERONE--434	10.731	0.796	48104	796.0
ETIOCHOLANOLONE--434	10.978	0.814	120160	1976.8
Mono-Androsterone--272	9.225	0.684	1765	
TESTOSTERONE--432	13.504	1.002	7700	28.7
EPITESTOSTERONE--432	12.653	0.938	5003	20.4
DHT=DIHYDROTESTOSTERONE--434	12.860	0.954	90	1.3
5a-Androstan-3a,17B-diol--241	11.122	0.825	2157	25.9
5B-Androstan-3a,17B-diol--241	11.266	0.836	9878	136.7
11-OH-ANDROST--522	13.872	1.029	24728	2026.1
11-OH-ETIOCHO--522	14.132	1.048	10439	1005.2
DHEA--432	12.133	0.900	2901	
VIT E METABOLITE--422	9.225	0.684	135748	
CORTISOL METAB --462	17.489	1.297	4063	

\* Height shown is d3T - 1% d0-T Peakheight.

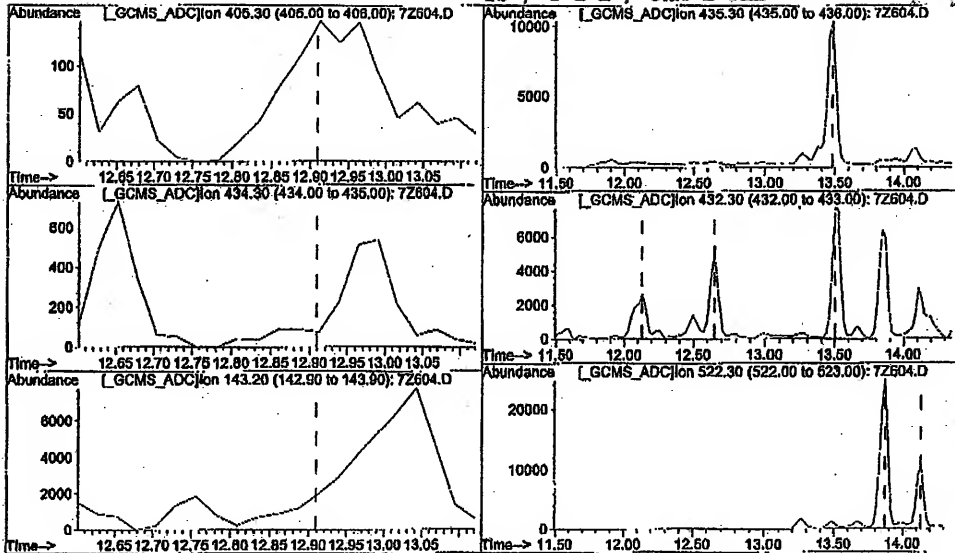
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8512
EIS 462-D3T	3.9100-4.1100	4.0070
D3T-VIT E MET	4.1600-4.3600	4.2571

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

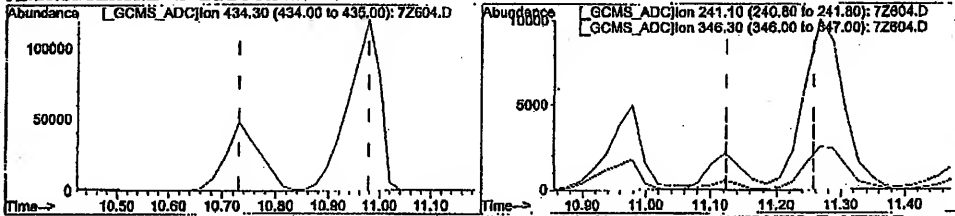
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.54
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.4
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	2.4
ANDROSTERONE	/ EPITESTOSTERONE	9.6
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	3.7 %
DHT	/ EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.3
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 3.6
D0-Testosterone	/ D3-Testosterone	0.7

## Analysis Report for Data File = 7Z604.D Graphics Page 1

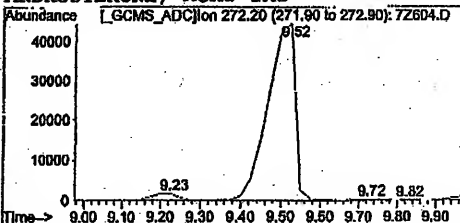
IS / T &amp; E / OHA &amp; OHE



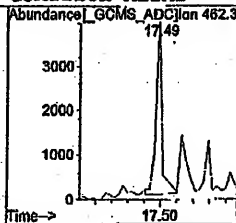
## ANDROSTERONE &amp; ETIOCHOLANOLONE



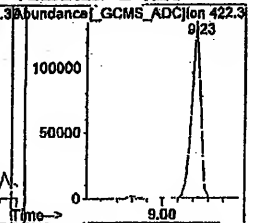
## ANDROSTERONE, Mono-TMS



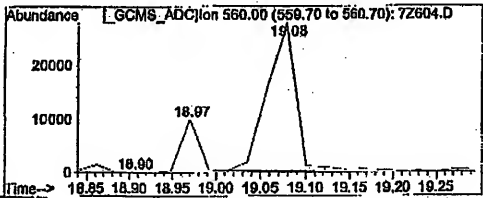
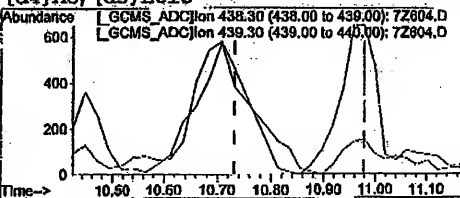
## CORTISOL, METAB



## VITAMIN E, METAB



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 7Y9 B/W 7Y9  
Miscellaneous: WU:A 2.5 mL NH4I RD/RJ  
Analysis Time: 3 Mar 2006 1:42 pm  
Method File : ANABO04

Data File: 7Z6101.D

Equipment # : MSDA8  
ALS Bottle # : 43

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.463	1.000	6476 *	<40.0>
ANDROSTERONE--434	10.733	0.797	61560	1620.8
ETIOCHOLANOLONE--434	10.937	0.812	79432	2079.4
Mono-Androsterone--272	9.225	0.685	2179	
TESTOSTERONE--432	13.484	1.002	5837	34.6
EPITESTOSTERONE--432	12.627	0.938	3406	22.1
DHT-DIHYDROTESTOSTERONE--434	12.835	0.953	111	2.6
5a-Androstan-3a,17B-diol--241	11.102	0.825	1974	37.7
5B-Androstan-3a,17B-diol--241	11.246	0.835	9309	205.0
11-OH-ANDROST--522	13.831	1.027	20824	2715.0
11-OH-ETIOCHO--522	14.091	1.047	9434	1445.4
DHEA--432	12.107	0.899	2145	
VIT E METABOLITE--422	9.225	0.685	259680	
CORTISOL METAB --462	17.489	1.299	1793	

\* Height shown is d3T - 1% d0-T Peakheight.

&gt;&gt; QC TIME RANGE FOUND &lt;&lt;

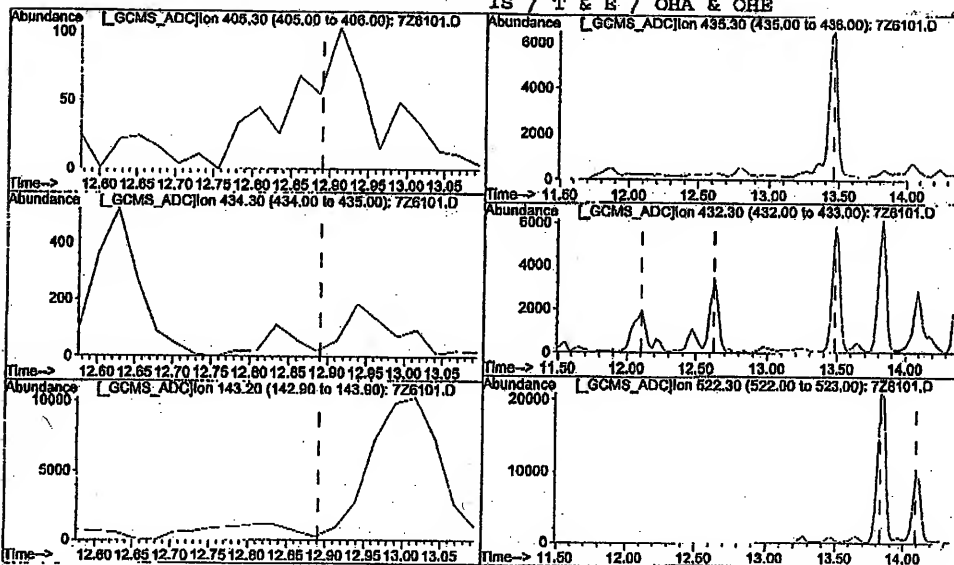
TEST-EPIT	0.8250-0.9150	0.8575
EIS 462-D3T	3.9100-4.1100	4.0261
D3T-VIT E MET	4.1600-4.3600	4.2378

&gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

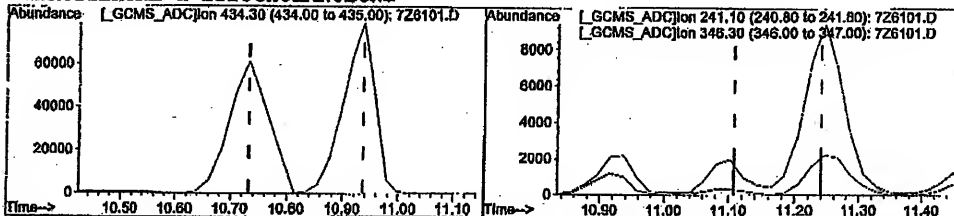
TESTOSTERONE	/	EPITESTOSTERONE (4)	1.71
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	0.8
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	2.2
ANDROSTERONE	/	EPITESTOSTERONE	18.1
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	3.6 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	1.7
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 6.3
D0-Testosterone	/	D3-Testosterone	0.9

## Analysis Report for Data File = 7Z6101.D Graphics Page 1

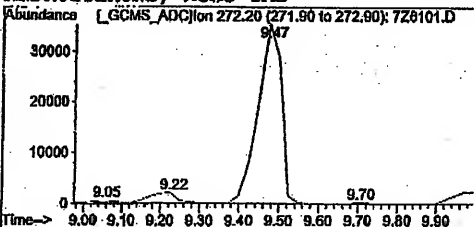
IS / T &amp; E / OHA &amp; OHE



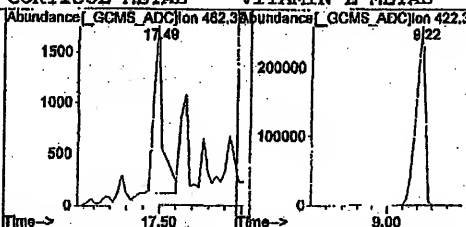
## ANDROSTERONE &amp; ETIOCHOLANOLONE



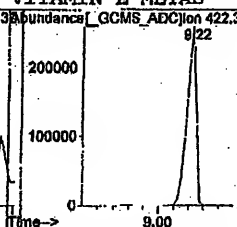
## ANDROSTERONE, Mono-TMS



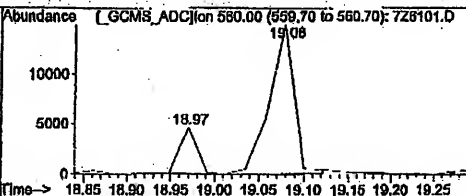
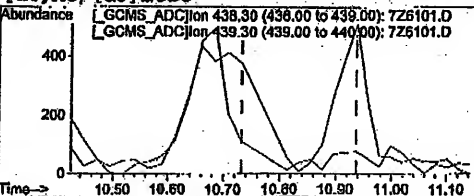
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Etio





PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

951872  
2/26/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 7Y9 B/W 7Y9  
Miscellaneous: WU:A 2.5 mL NH4I RD/RJ  
Analysis Time: 3 Mar 2006 2:06 pm  
Method File : ANABO04

Data File: 7Z6111.D

Equipment # : MSDA8  
ALS Bottle # : 44

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.439	1.000	8300 *	<40.0>
ANDROSTERONE--434	10.707	0.797	53832	1105.7
ETIOCHOLANOLONE--434	10.916	0.812	66544	1359.0
Mono-Androsterone--272	9.275	0.690	82	
TESTOSTERONE--432	13.482	1.003	4356	20.1
EPITESTOSTERONE--432	12.601	0.938	2387	12.1
DHT-DIHYDROTESTOSTERONE--434	12.835	0.955	80	1.5
5a-Androstan-3a,17B-diol--241	11.081	0.825	920	13.7
5B-Androstan-3a,17B-diol--241	11.225	0.835	4958	85.2
11-OH-ANDROST--522	13.829	1.029	12799	1301.9
11-OH-ETIOCHO--522	14.089	1.048	5119	611.9
DHEA--432	12.081	0.899	1004	
VIT E METABOLITE--422	9.175	0.683	94220	
CORTISOL METAB --462	17.462	1.299	1361	

\* Height shown is d3T - 1% d0-T Peakheight.

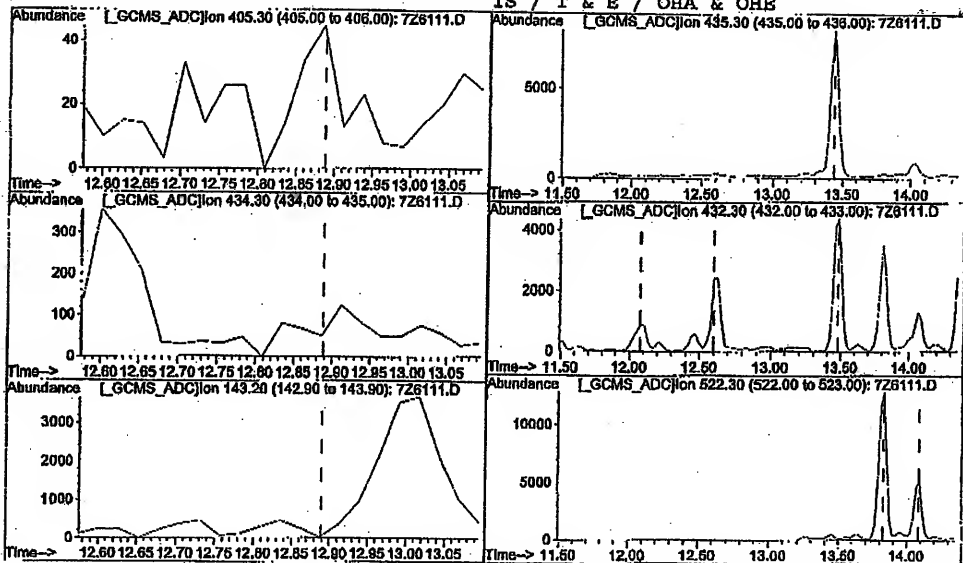
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8812
EIS 462-D3T	3.9100-4.1100	4.0230
D3T-VIT E MET	4.1600-4.3600	4.2639

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

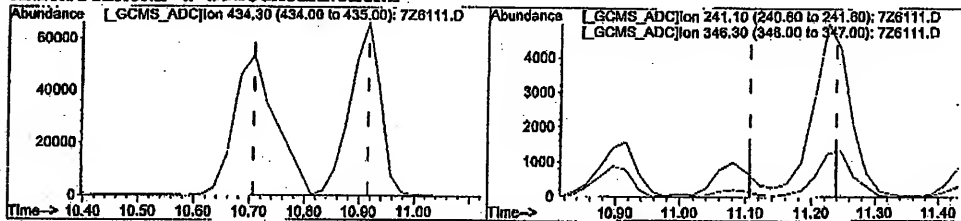
TESTOSTERONE	/	EPITESTOSTERONE (4)	1.82
ANDROSTERONE	/	ETIOCHOLANOLONE (3)	0.8
OH-ANDROSTERONE	/	OH-ETIOCHOLANOLONE	2.5
ANDROSTERONE	/	EPITESTOSTERONE	22.6
MONO-ANDROSTERONE	/	DI-ANDROSTERONE (5)	0.2 %
DHT	/	EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/	EPITESTOSTERONE (10)	1.1
5a-A-3a,17B-DIOL	/	5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/	D5-Etio (0.7 - 1.2)	*** 3.5
D0-Testosterone	/	D3-Testosterone	0.5

## Analysis Report for Data File = 7Z6111.D Graphics Page 1

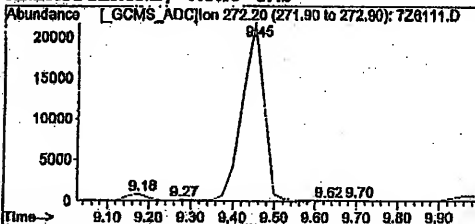
IS / T &amp; E / OHA &amp; OHE



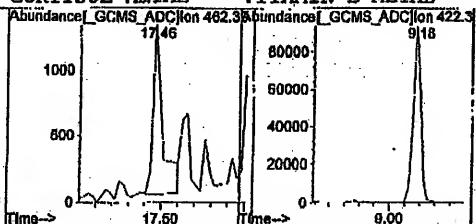
## ANDROSTERONE &amp; ETIOCHOLANOLONE



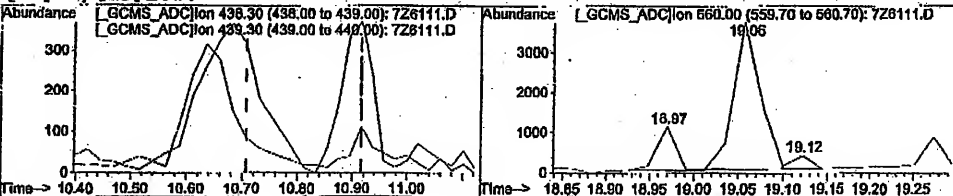
## ANDROSTERONE, Mono-TMS



## CORTISOL METAB VITAMIN E METAB



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

1504999  
4/10/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 8YZ  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 19 Apr 2006 11:28 am  
Method File : ANABO04

Data File: 8YZ08.D  
Equipment # : MSDA11  
ALS Bottle # : 32

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.461	1.000	32866 *	<40.0>
ANDROSTERONE--434	10.739	0.798	303360	1524.9
ETIOCHOLANOLONE--434	11.033	0.820	762560	3744.6
Mono-Androsterone--272	9.507	0.706	102524	
TESTOSTERONE--432	13.504	1.003	43035	53.6
EPITESTOSTERONE--432	12.634	0.939	42235	53.0
DHT-DIHYDROTESTOSTERONE--434	12.839	0.954	986	3.9
5a-Androstan-3a,17B-diol--241	11.123	0.826	15554	47.8
5B-Androstan-3a,17B-diol--241	11.258	0.836	90273	398.3
11-OH-ANDROST--522	13.847	1.029	66160	434.5
11-OH-ETIOCHO--522	14.104	1.048	46024	361.8
DHEA--432	12.119	0.900	14909	
VIT E METABOLITE--422	9.185	0.682	190026	
CORTISOL METAB --462	17.510	1.301	63465	

\* Height shown is d3T - 1% d0-T Peakheight.

>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8701
EIS 462-D3T	3.9100-4.1100	4.0486
D3T-VIT E MET	4.1600-4.3600	4.2759

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

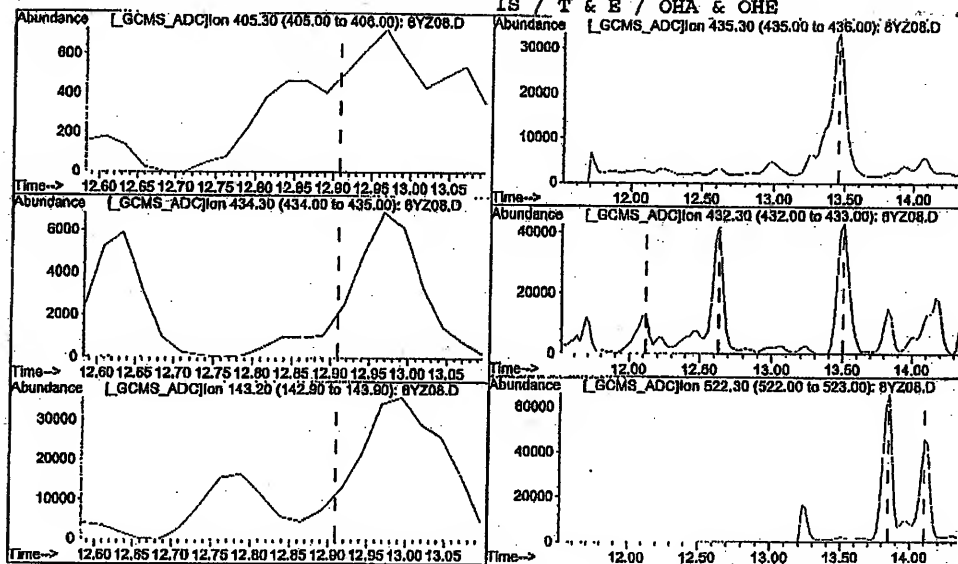
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.02
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.4
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	1.4
ANDROSTERONE	/ EPITESTOSTERONE	7.2
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	*** 33.8 % - not mono (linear done)
DHT	/ EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	0.9
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.1
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 1.7
D0-Testosterone	/ D3-Testosterone	1.3

WR  
DTC 3/30/07

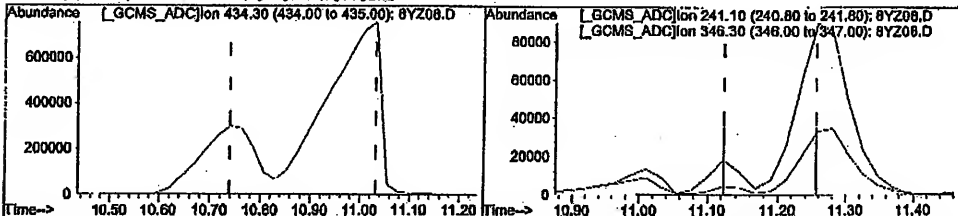
!! The Androsterone Mono-TMS is > 5% of Di-TMS in this sample !!

## Analysis Report for Data File = 8YZ08.D Graphics Page 1

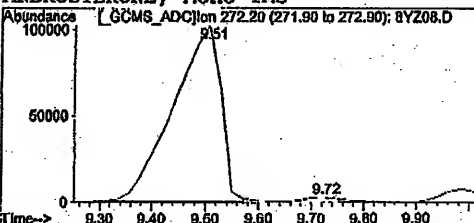
IS / T &amp; E / OHA &amp; OHE



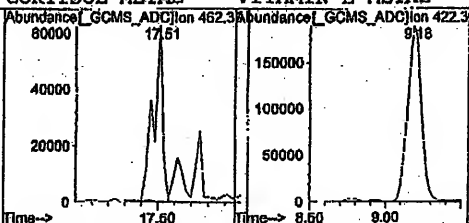
## ANDROSTERONE &amp; ETIOCHOLANOLONE



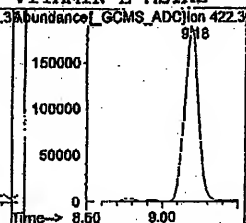
## ANDROSTERONE, Mono-TMS



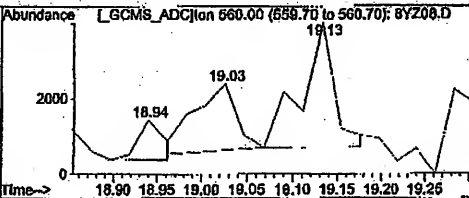
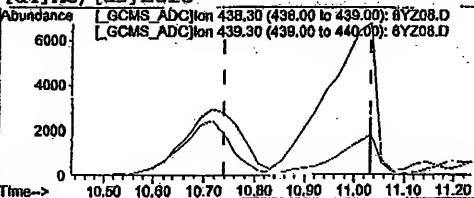
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

951789  
4/21/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 88R  
Miscellaneous: WU:A 2.5 mL NH4I RD/RJ  
Analysis Time: 2 May 2006 10:58 am  
Method File : ANABO04

Data File: 88R111.D

Equipment # : MSDA10  
ALS Bottle # : 41

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.449	1.000	20680 *	<40.0>
ANDROSTERONE--434	10.752	0.799	171712	2425.2
ETIOCHOLANOLONE--434	10.979	0.816	207424	3018.1
Mono-Androsterone--272	9.335	0.694	755	
TESTOSTERONE--432	13.492	1.003	21556	51.8
EPITESTOSTERONE--432	12.622	0.938	12195	34.0
DHT-DIHYDROTESTOSTERONE--434	12.956	0.963	3000	16.1
5a-Androstan-3a,17B-diol--241	11.114	0.826	6351	37.3
5B-Androstan-3a,17B-diol--241	11.249	0.836	19200	138.6
11-OH-ANDROST--522	13.856	1.030	91576	981.7
11-OH-ETIOCHO--522	14.114	1.049	44064	528.5
DHEA--432	12.108	0.900	7896	
VIT E METABOLITE--422	9.249	0.688	274251	
CORTISOL METAB --462	17.444	1.297	19007	

\* Height shown is d3T - 1% d0-T Peakheight.

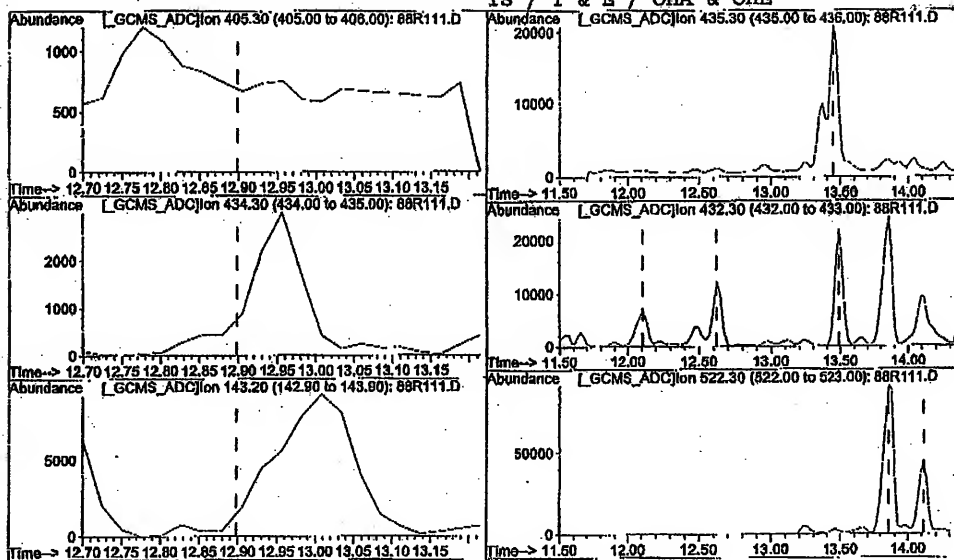
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8701
EIS 462-D3T	3.9100-4.1100	3.9951
D3T-VIT E MET	4.1600-4.3600	4.1996

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

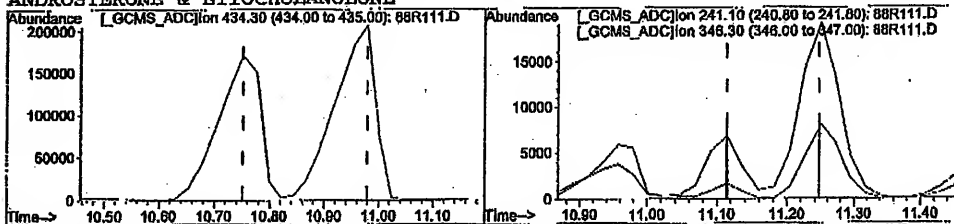
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.77
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.8
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	2.1
ANDROSTERONE	/ EPITESTOSTERONE	14.1
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	0.4 %
DHT	/ EPITESTOSTERONE (1.8)	0.5
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.1
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 6.1
D0-Testosterone	/ D3-Testosterone	1.0

## Analysis Report for Data File - 88R111.D Graphics Page 1

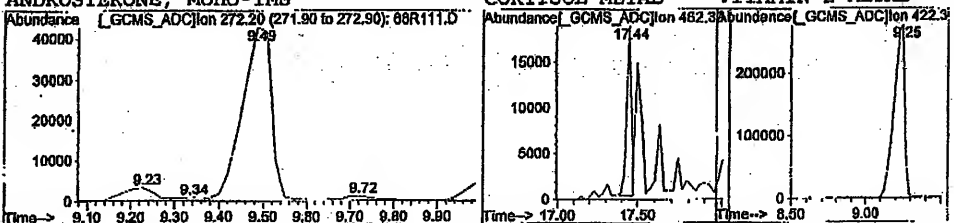
IS / T &amp; E / OHA &amp; OHE



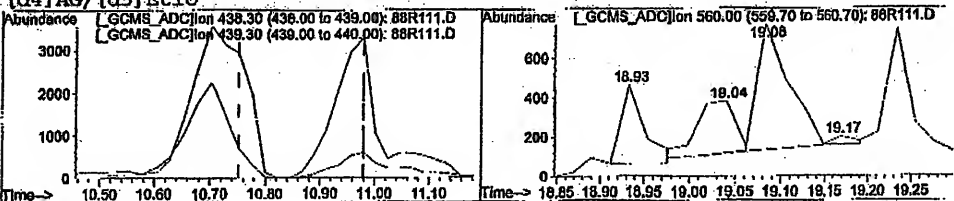
## ANDROSTERONE &amp; ETIOCHOLANOLONE



## ANDROSTERONE, Mono-TMS



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

951792  
4/20/06

Sample Name : 83P B/W 83P  
Miscellaneous: WU:A 2.5 mL NH4I PENTANE RW  
Analysis Time: 8 May 2006 5:45 pm  
Method File : ANAB004

Data File: 88R17B.D  
Equipment # : MSDA14  
ALS Bottle # : 11

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.386	1.000	36496 *	<40.0>
ANDROSTERONE--434	10.695	0.799	449600	1812.6
ETIOCHOLANOLONE--434	10.908	0.815	592384	2335.0
Mono-Androsterone--272	9.274	0.693	1629	
TESTOSTERONE--432	13.407	1.002	42401	44.3
EPITESTOSTERONE--432	12.572	0.939	17881	24.9
DHT-DIHYDROTESTOSTERONE--434	12.777	0.955	493	1.8
5a-Androstan-3a,17b-diol--241	11.051	0.826	9542	24.2
5b-Androstan-3a,17b-diol--241	11.194	0.836	42461	182.0
11-OH-ANDROST--522	13.772	1.029	123976	1111.0
11-OH-ETIOCHO--522	14.051	1.050	111008	1674.1
DHEA--432	12.032	0.899	10426	
VIT E METABOLITE--422	9.145	0.683	1173	
CORTISOL METAB --462	17.411	1.301	15224	

\* Height shown is d3T - 1% d0-T Peakheight.

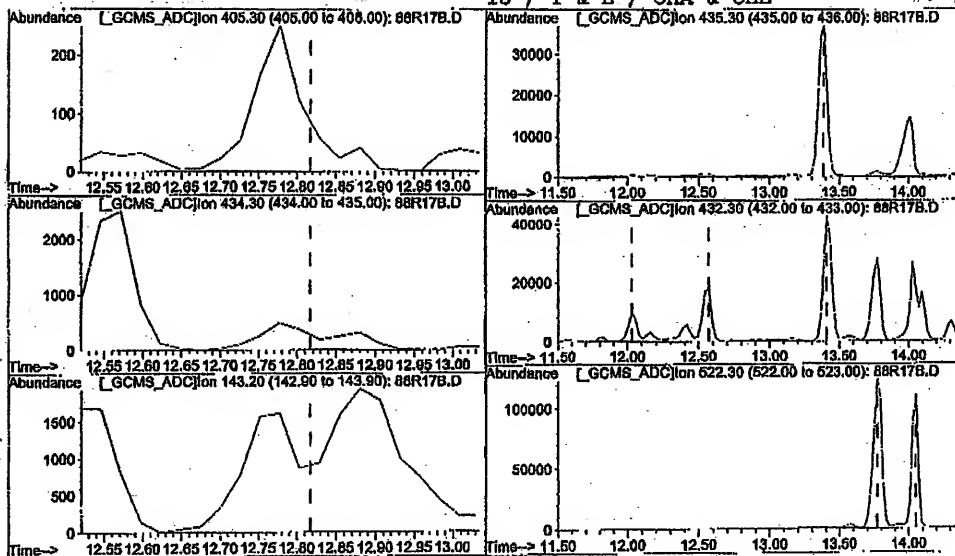
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8357
EIS 462-D3T	3.9100-4.1100	4.0245
D3T-VIT E MET	4.1600-4.3600	4.2410

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

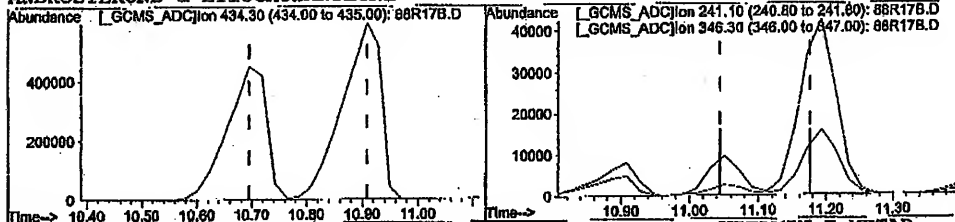
TESTOSTERONE	/ EPITESTOSTERONE (4)	2.37
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.8
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	1.1
ANDROSTERONE	/ EPITESTOSTERONE	25.1
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	0.4 %
DHT	/ EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.0
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.1
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 5.2
D0-Testosterone	/ D3-Testosterone	1.2

## Analysis Report for Data File = 88R17B.D Graphics Page 1

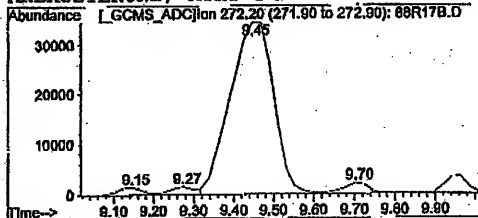
IS / T &amp; E / OHA &amp; OHE



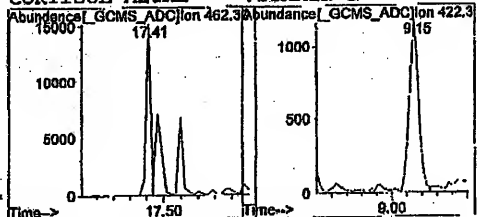
## ANDROSTERONE &amp; ETIOCHOLANOLONE



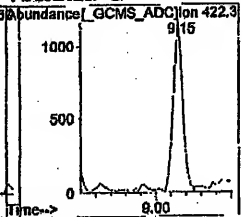
## ANDROSTERONE, Mono-TMS



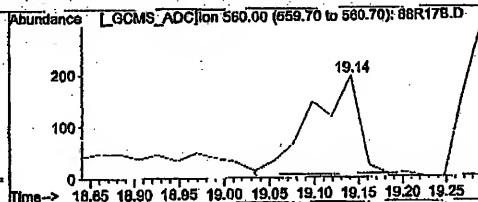
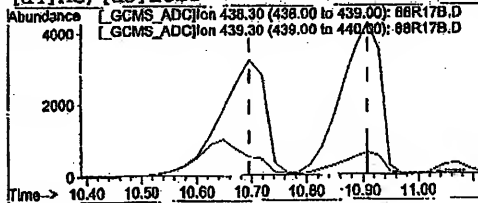
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Etio





PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

951787  
4/22/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 88R  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 1 May 2006 11:56 pm  
Method File : ANAB004

Data File: 88R19.D  
Equipment # : MSDA10  
ALS Bottle # : 49

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.492	1.000	10142 *	<40.0>
ANDROSTERONE--434	10.821	0.802	61840	1781.0
ETIOCHOLANOLONE--434	11.047	0.819	77792	2308.1
Mono-Androsterone--272	9.271	0.687	2167	
TESTOSTERONE--432	13.535	1.003	11437	56.0
EPITESTOSTERONE--432	12.673	0.939	7477	42.5
DHT-DIHYDROTESTOSTERONE--434	13.007	0.964	3274	35.8
5a-Androstan-3a,17B-diol--241	11.159	0.827	4987	59.7
5B-Androstan-3a,17B-diol--241	11.294	0.837	23968	352.8
11-OH-ANDROST--522	13.899	1.030	29488	644.6
11-OH-ETIOCHO--522	14.178	1.051	25856	632.4
DHEA--432	12.159	0.901	4350	
VIT E METABOLITE--422	9.292	0.689	109657	
CORTISOL METAB --462	17.471	1.295	9724	

\* Height shown is d3T - 1% d0-T Peakheight.

>> QC TIME RANGE FOUND <<

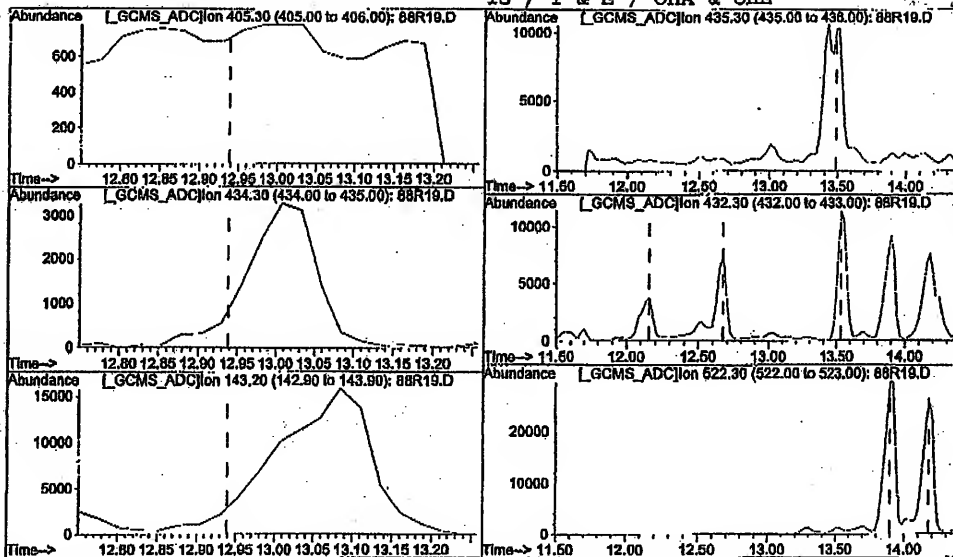
TEST-EPIT	0.8250-0.9150	0.8615
EIS 462-D3T	3.9100-4.1100	3.9790
D3T-VIT E MET	4.1600-4.3600	4.1996

>> RATIOS (CUT OFF VALUE) AND QUANTITATION <<

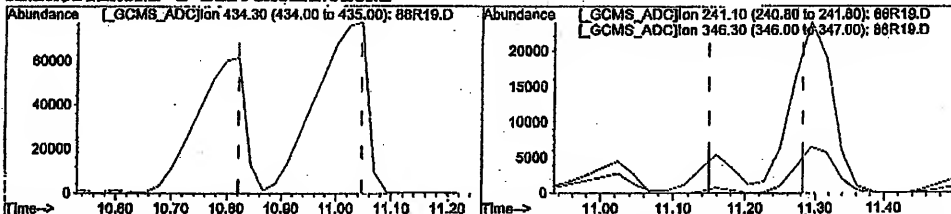
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.53
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.8
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	1.1
ANDROSTERONE	/ EPITESTOSTERONE	8.3
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	3.5 %
DHT	/ EPITESTOSTERONE (1.8)	0.8
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.4
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 5.4
D0-Testosterone	/ D3-Testosterone	1.1

## Analysis Report for Data File = 88R19.D Graphics Page 1

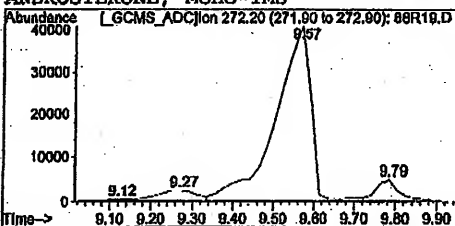
IS / T &amp; E / OHA &amp; OHE



## ANDROSTERONE &amp; ETIOCHOLANOLONE

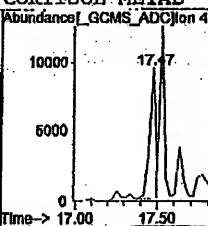


## ANDROSTERONE, Mono-TMS

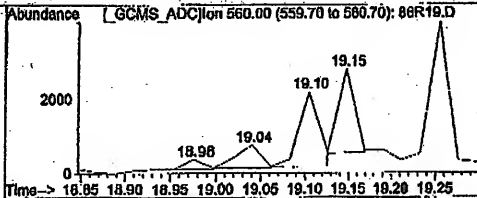
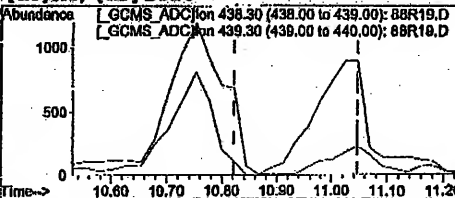
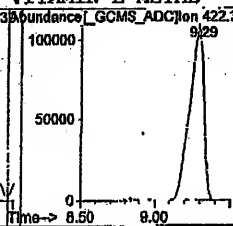


[d4]Ag / [d5]Et10

## CORTISOL METAB



## VITAMIN E METAB



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 88R  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 2 May 2006 1:41 am  
Method File : ANAB004

Data File: 88R23.D  
Equipment # : MSDA10  
ALS Bottle # : 53

95770  
4/23/06  
OK  
2/24/07

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.492	1.000	24483 *	<40.0>
ANDROSTERONE--434	10.822	0.802	70864	845.4
ETIOCHOLANOLONE--434	11.024	0.817	75184	924.0
Mono-Androsterone--272	9.249	0.686	2410	
TESTOSTERONE--432	13.535	1.003	33308	67.6
EPITESTOSTERONE--432	12.673	0.939	23580	55.5
DHT-DIHYDROTESTOSTERONE--434	12.802	0.949	123	0.6
5a-Androstan-3a,17B-diol--241	11.159	0.827	5549	27.5
5B-Androstan-3a,17B-diol--241	11.294	0.837	26395	160.9
11-OH-ANDROST--522	13.921	1.032	124072	1123.5
11-OH-ETIOCHO--522	14.178	1.051	67456	683.4
DHEA--432	12.159	0.901	4077	
VIT E METABOLITE--422	9.292	0.689	107790	
CORTISOL METAB --462	17.471	1.295	27480	

\* Height shown is d3T - 1% d0-T Peakheight.

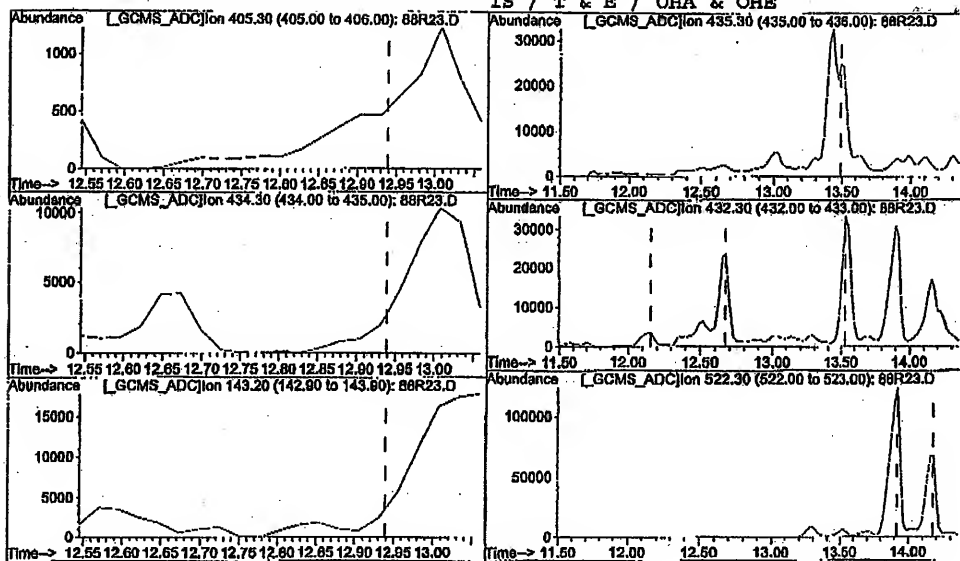
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8616
EIS 462-D3T	3.9100-4.1100	3.9790
D3T-VIT E MET	4.1600-4.3600	4.1996

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

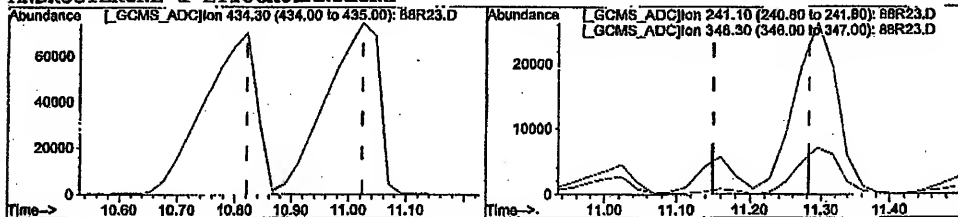
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.41
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.9
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	1.8
ANDROSTERONE	/ EPITESTOSTERONE	3.0
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	3.4 %
DHT	/ EPITESTOSTERONE (1.8)	0.0
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	0.5
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.2
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 35.5
D0-Testosterone	/ D3-Testosterone	1.4

## Analysis Report for Data File = 88R23.D Graphics Page 1

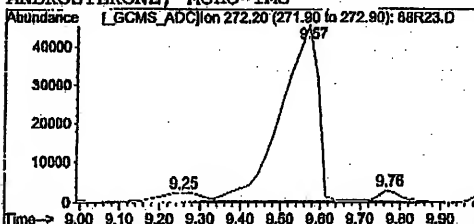
IS / T &amp; E / OHA &amp; OHE



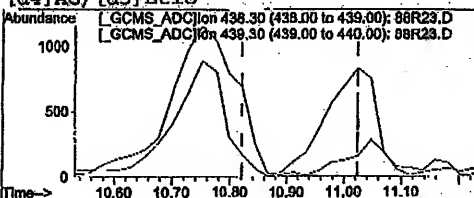
## ANDROSTERONE &amp; ETIOCHOLANOLONE



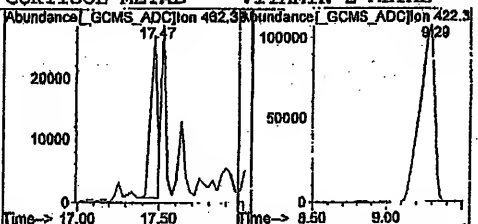
## ANDROSTERONE, Mono-TMS



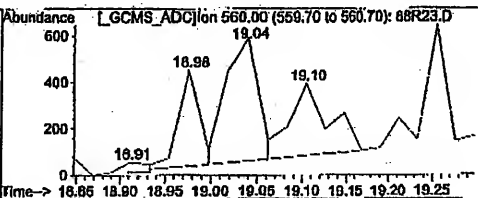
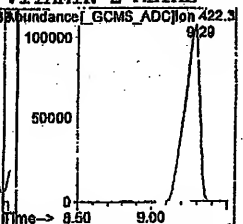
## [d4]AG/[d5]Etio



## CORTISOL METAB



## VITAMIN E METAB



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

1501850  
8/3/06

Sample Name : 94T  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 6 Aug 2006 6:51 am  
Method File : ANAB004

Data File: 94T03.D  
Equipment # : MSDA11  
ALS Bottle # : 80

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.397	1.000	34468 *	<40.0>
ANDROSTERONE--434	10.692	0.798	103584	532.4
ETIOCHOLANOLONE--434	10.898	0.813	245760	1284.4
Mono-Androsterone--272	9.271	0.692	221	
TESTOSTERONE--432	13.440	1.003	7620	8.3
EPITESTOSTERONE--432	12.582	0.939	4947	5.7
DHT=DIHYDROTESTOSTERONE--434	12.788	0.955	238	1.0
5a-Androstan-3a,17B-diol--241	11.055	0.825	3079	7.1
5B-Androstan-3a,17B-diol--241	11.190	0.835	27391	88.5
11-OH-ANDROST--522	13.804	1.030	37896	186.6
11-OH-ETIOCHO--522	14.040	1.048	9080	46.5
DHEA--432	12.042	0.899	4206	
VIT E METABOLITE--422	9.142	0.682	33233	
CORTISOL METAB --462	17.510	1.307	6772	

\* Height shown is d3T - 1% d0-T Peakheight.

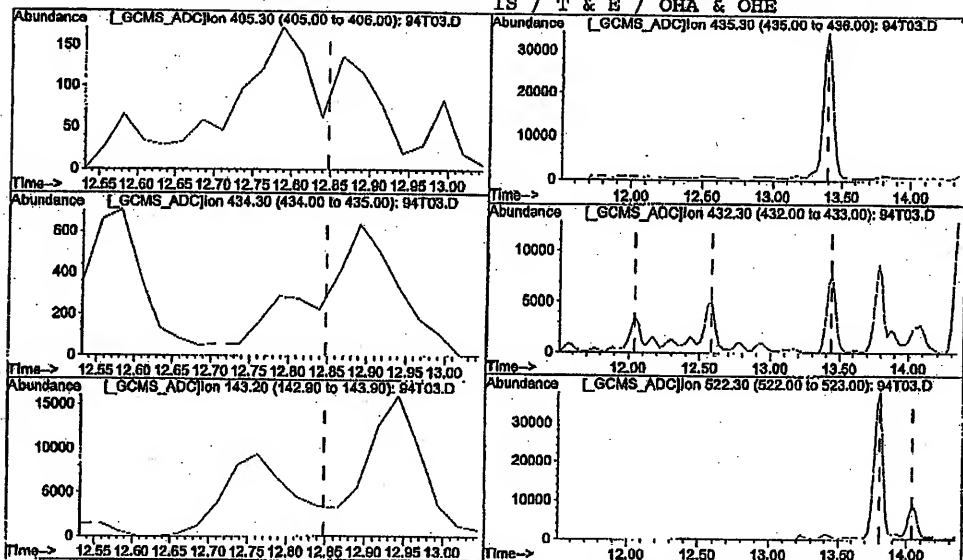
>> QC	TIME RANGE	FOUND <<
TEST-EPIT	0.8250-0.9150	0.8572
EIS 462-D3T	3.9100-4.1100	4.1130
D3T-VIT E MET	4.1600-4.3600	4.2544

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

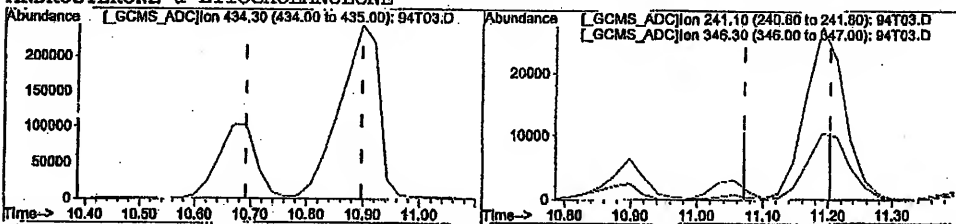
TESTOSTERONE	/ EPITESTOSTERONE (4)	1.54
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.4
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	4.2
ANDROSTERONE	/ EPITESTOSTERONE	20.9
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	0.2 %
DHT	/ EPITESTOSTERONE (1.8)	0.2
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	1.2
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.1
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 2.9
D0-Testosterone	/ D3-Testosterone	0.2

## Analysis Report for Data File = 94T03.D Graphics Page 1

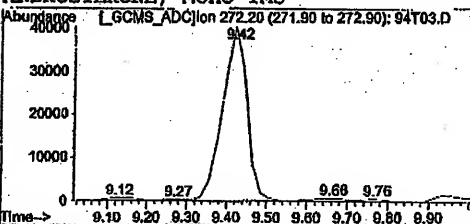
IS / T &amp; E / OHA &amp; OHE



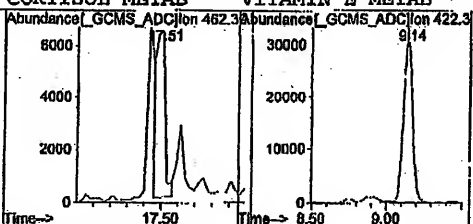
## ANDROSTERONE &amp; ETIOCHOLANOLONE



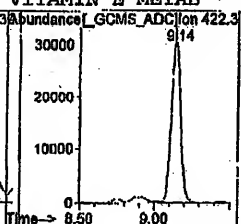
## ANDROSTERONE, Mono-TMS



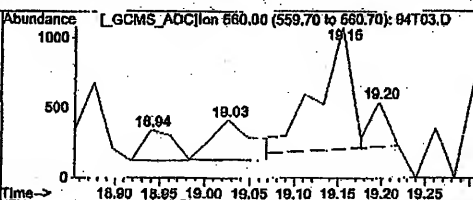
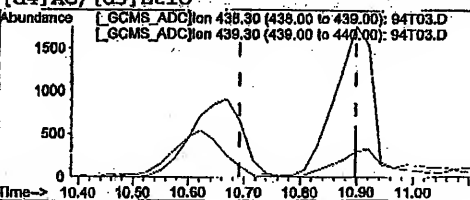
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Etio



PAUL ZIFFREN OLYMPIC ANALYTICAL LABORATORY  
UCLA SCHOOL OF MEDICINE

497104  
8/21/06

## &gt;&gt;&gt; Analysis Report &lt;&lt;&lt;

Sample Name : 9S6  
Miscellaneous: WU:A 2.5 mL NH4I  
Analysis Time: 1 Sep 2006 8:51 pm  
Method File : ANABO04

Data File: 9S604.D

Equipment # : MSDA8  
ALS Bottle # : 20

Compound	RT	RRT	Peak Height	Conc. (ng/mL)
I.S. TRIDEUTERATED T.--435	13.527	1.000	24875 *	<40.0>
ANDROSTERONE--434	10.854	0.802	523072	5444.5
ETIOCHOLANOLONE--434	11.121	0.822	760192	10163.0
Mono-Androsterone--272	9.226	0.682	6556	
TESTOSTERONE--432	13.570	1.003	43683	73.2
EPITESTOSTERONE--432	12.704	0.939	46659	83.4
DHT-DIHYDROTESTOSTERONE--434	12.912	0.955	1683	8.2
5a-Androstan-3a,17B-diol--241	11.204	0.828	28173	271.3
5B-Androstan-3a,17B-diol--241	11.348	0.839	149694	870.8
11-OH-ANDROST--522	13.960	1.032	238976	1291.2
11-OH-ETIOCHO--522	14.199	1.050	91064	703.6
DHEA--432	12.184	0.901	18968	
VIT E METABOLITE--422	9.251	0.684	168000	
CORTISOL METAB --462	17.490	1.293	28729	

\* Height shown is d3T - 1% d0-T Peakheight.

## &gt;&gt; QC TIME RANGE FOUND &lt;&lt;

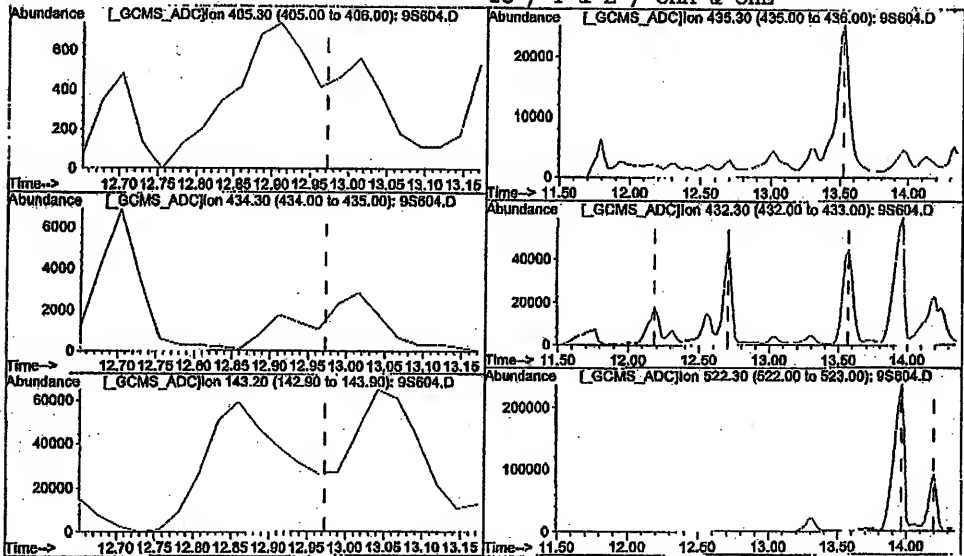
TEST-EPIT	0.8250-0.9150	0.8663
EIS 462-D3T	3.9100-4.1100	3.9634
D3T-VIT E MET	4.1600-4.3600	4.2763

## &gt;&gt; RATIOS (CUT OFF VALUE) AND QUANTITATION &lt;&lt;

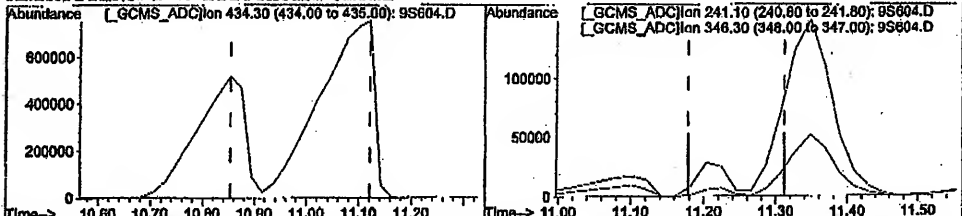
TESTOSTERONE	/ EPITESTOSTERONE (4)	0.94
ANDROSTERONE	/ ETIOCHOLANOLONE (3)	0.7
OH-ANDROSTERONE	/ OH-ETIOCHOLANOLONE	2.6
ANDROSTERONE	/ EPITESTOSTERONE	11.2
MONO-ANDROSTERONE	/ DI-ANDROSTERONE (5)	1.3 %
DHT	/ EPITESTOSTERONE (1.8)	0.1
5a-A-3a,17B-DIOL	/ EPITESTOSTERONE (10)	3.3
5a-A-3a,17B-DIOL	/ 5b-A-3a,17B-DIOL (3)	0.3
D4-Andro-gluc	/ D5-Etio (0.7 - 1.2)	*** 2.2
D0-Testosterone	/ D3-Testosterone	1.8

## Analysis Report for Data File = 9S604.D Graphics Page 1

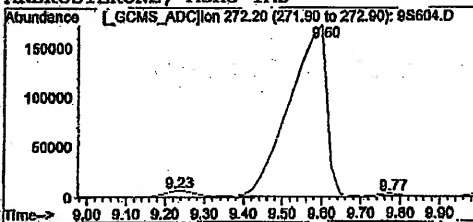
IS / T &amp; E / OHA &amp; OHE



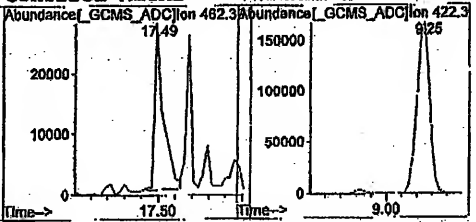
## ANDROSTERONE &amp; ETIOCHOLANOLONE



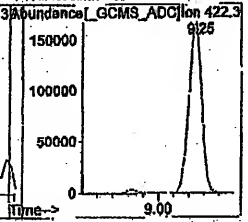
## ANDROSTERONE, Mono-TMS



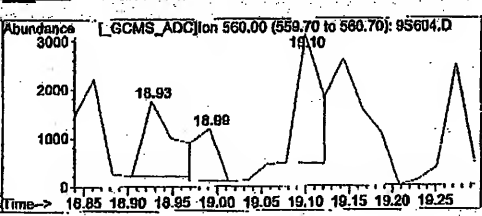
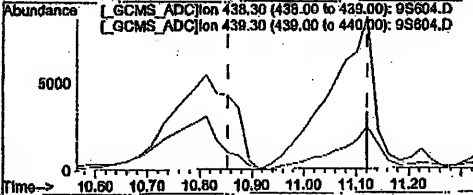
## CORTISOL METAB



## VITAMIN E METAB



## [d4]AG/[d5]Etio





# CONFIDENTIAL

Page 1 of 1

**Travis Tygart**

---

**From:** Don Catlin [dcatlin@ucla.edu]  
**Sent:** Monday, January 08, 2007 6:08 PM  
**To:** Richard Young; Travis Tygart  
**Subject:** interesting data

These data are from a study that I have been working on for the last few years. Testosterone was infused at 0 (baseline), 7, 14, or 28 mg/2 sq m of BSA/24 hours. Urines were collected at the intervals shown on the graph.

Delta delta relative to the pregnanediol is plotted for the 5a and 5b diols in black and white diamonds, respectively. The difference between the delta deltas for these 2 compounds is plotted using the dashed line. The bold line at approximately -4 corresponds to the difference seen in a USADA cycling case.

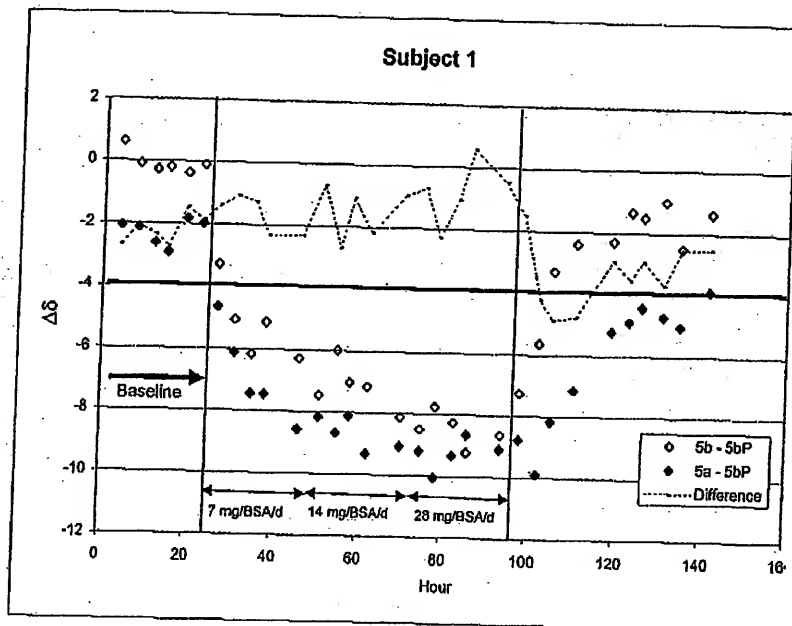
For this particular subject it is apparent that between hours 100 and 120 which corresponds to the time period after infusion ceased the data approach those seen in the USADA case where delta deltas for the 5a and 5b diols were -6.1 and -2.2, respectively.

Dhc

1/9/2007

USADA1133

Please fax to Rod Young + Travis Tye



CONFIDENTIAL



Agence Française de l'Info Drogue  
Département des Analyses

Châtenay-Malabry, the 25<sup>th</sup> January 2007

## TRANSMISSION DE TELECOPIE

<p><b>Expéditeur :</b> J. de CEARRIZ Directeur du Département des Analyses</p> <p><b>Tél :</b> +33 (0) 1.46.60.28.69 <b>Fax :</b> +33 (0) 1.46.60.30.17 <b>e-mail :</b> Analyses@afid.fr</p>	<p><b>Destinataire :</b> Travis Tygart <b>Organisme :</b> USADA</p> <p><b>Fax :</b> 001.719.785.2001</p>
--	--

Nombre de pages y compris celle-ci : 2

Dear Travis Tygart,

Please, find here a new proposal from our laboratory for the IRMS analysis of samples n° 995462 B, n° 994203 B, n° 994277 B, n° 994276 B, n° 994075 B, n° 994080 B, n° 994171 B, from Tour de France 2006 and IRMS analysis of the two additional B samples from USADA n° 497104 B = UCLA 95604 and n° 1501850 B = UCLA 94T03 in February 2007

The B analysis may be performed from February 5 to February 23, 2007. A more detailed program was attached to this fax letter.

Yours Sincerely,

J. de CEARRIZ

**February 5 to 9, 2007**

	February 5 Monday	February 6 Tuesday	February 7 Wednesday	February 8 Thursday	February 9 Friday
Opening at 9h00 a.m	B 995462 B 994203	B 994277 B 994276			
Sample extraction and derivatization	B 995462 B 994203	B 995462 B 994203 B 994277 B 994276	B 994277 B 994276		
GC-MS and IRMS analysis			B 995462 B 994203	B 994277 B 994276 B 995462 B 994203	B 994277 B 994276
Result available					

**February 12 to 16, 2007**

	February 12 Monday	February 13 Tuesday	February 14 Wednesday	February 15 Thursday	February 16 Friday
Opening at 9h00 a.m	UCLA 9S604 B 994075	B 994080			
Sample extraction and derivatization	UCLA 9S604 B 994075	UCLA 9S604 B 994080	B 994080		
GC-MS and IRMS analysis			UCLA 9S604 B 994075	B 994080 UCLA 9S604 B 994075	B 994080
Result available					

**February 19 to 23, 2007**

	February 19 Monday	February 20 Tuesday	February 21 Wednesday	February 22 Thursday	February 23 Friday
Opening at 9h00 a.m	B 994171 UCLA 94T03				
Sample extraction and derivatization	B 994171 UCLA 94T03	B 994171 UCLA 94T03			
GC-MS and IRMS analysis			B 994171 UCLA 94T03		
Result available				B 994171 UCLA 94T03	

**aflid**Agence Française de l'Anti-Dopage  
Département des AnalysesChâtenay-Malabry, the 1<sup>st</sup> February, 2007**TRANSMISSION DE TELECOPIE**

<b>Expéditeur :</b>  J. de CEARRIZ Directeur du Département des Analyses  <b>Tél :</b> +33 (0) 1.46.60.28.69 <b>Fax :</b> +33 (0) 1.46.60.38.17 <b>e-mail :</b> Analyses@aflid.fr	<b>Destinataire :</b>  Travis T. Tygart <b>Organisme :</b>  USADA  <b>Fax :</b> 00.1.719.785.2001
--	--

Nombre de pages y compris celle-ci : 2

Dear Travis Tygart,

I confirm the receipt of your request to temporarily postpone testing previously scheduled to commence February 5, 2007.

We are waiting your decision before rescheduling the testing.

Yours Sincerely,

J. de CEARRIZ  


**TAB 47**



**VIA OVERNIGHT MAIL and VIA E-MAIL and VIA FACSIMILE**

July 27, 2006

Mr. Floyd A. Landis  
23356 Bishop Road  
Murrieta, CA 92542

Michael P. Rutherford, Esq.  
1435 Stuart Street  
Denver, CO 80204

RE: UCI File No. 29/06  
Tour de France, July 20, 2006  
Sample #995474

**Board of Directors**

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Andrew Mecca, Dr PH, MPH

Annette Solmeen, DPhil

Dear Mr. Landis:

Your urine sample collected at the Tour de France on July 20, 2006, was sent to the WADA accredited laboratory at Chateauf-Malabry, France, ("the Laboratory") for analysis. The Laboratory has reported that your A sample contains an elevated testosterone/epitestosterone (T/E) ratio at a prohibited level of greater than 4:1. Additionally, the laboratory performed a Carbon Isotope Ratio (CIR) analysis on your sample and reported the result positive for exogenous testosterone or its precursors. The World Anti-Doping Agency's Prohibited List, adopted by both the USADA Protocol for Olympic Movement Testing ("Protocol") and the Union Cycliste Internationale ("UCI") Anti-Doping Rules, lists testosterone and its precursors as prohibited substances in the class of anabolic androgenic steroids.

I have enclosed the July 26, 2006, letter from UCI and the documents attached to that letter, including the Laboratory's report, referring your case. On July 26, 2006, USA Cycling forwarded the UCI documents to USADA and in accordance with the rules requested that USADA handle the adjudication of this matter. I have also enclosed a copy of this correspondence from USA Cycling to USADA and USADA's response of today to USA Cycling formally accepting the handling of your case.

As referenced in the UCI letter dated July 26, 2006, in accordance with UCI rules you must immediately request, without delay, the B sample analysis or the B sample analysis will be deemed waived and the laboratory results conclusively established. In accordance with UCI rule 194, the request for the analysis of the B sample must be made no more than five (5) working days from July 26, 2006, the date of the UCI letter. Please inform me immediately in writing by fax at 719-785-2028 if you elect to have the B opening and analysis performed and we will notify the Laboratory and UCI of your request and attempt to schedule the date for the B analysis. You also

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have the right to attend the B sample opening and analysis if requested (which may take many hours, over multiple days).

Further, if you choose not to request the B sample analysis, USADA will forward your case immediately to a panel of the independent USADA Anti-Doping Review Board, as set forth in the USADA Protocol, for its consideration. Under the USADA Protocol and the UCI Anti-Doping Rules, the finding of a prohibited substance or method in an athlete's sample constitutes a doping violation. If it is ultimately determined that this is your first doping violation, a sanction may be imposed that will include disqualification of your competitive results achieved on and subsequent to July 20, 2006, the day your sample was collected, and up to a two year period of ineligibility.

Additionally, you have the right, at this time, to accept a "provisional suspension." By accepting a "provisional suspension," you will be immediately suspended from competing in all competitions under the jurisdiction of UCI, USA Cycling, and the United States Olympic Committee ("USOC"), or any of these entities' clubs, member associations or affiliates, until your case is deemed not to be a doping offense, you accept a sanction, you fail to contest this matter, or a hearing has been held in this matter. If you choose to accept this "provisional suspension," the time served under the "provisional suspension" will be deducted from any period of ineligibility that you might receive beginning on the date you accept the "provisional suspension" and notify USADA of such acceptance. If you do not to execute and return this "provisional suspension," any period of ineligibility you might receive will begin on the date of your acceptance of the sanction or on the date of the arbitration hearing panel's decision.

If you execute and return the "provisional suspension," USADA will give notice to the USOC, UCI, and USA Cycling of your acceptance of the "provisional suspension." Your decision to accept this "provisional suspension" is purely optional. You do not have to accept this "provisional suspension" in order to proceed with your case. If you are willing to accept a "provisional suspension," please inform us in writing immediately, by executing and returning the attached USADA Acceptance of Provisional Suspension Form.

If you intend to compete in any protected competitions, USADA has the right under its Protocol, Section 13, to expedite this matter to final resolution prior to the protected competition.



Also of importance, you are still subject to testing pending the outcome of this matter.

USADA will not publicly disclose or comment on the specifics of your test results until your case has been resolved. By copy of this letter, USADA is notifying USA Cycling and the USOC of your test results and requests that these organizations not comment publicly concerning this information until disclosed as provided in the USADA Protocol.

Enclosed for your reference are copies of the USADA Protocol, UCI Anti-Doping Rules and the World Anti-Doping Code, which set forth the administrative procedures followed for positive and elevated tests and other alleged anti-doping rule violations. You may also wish to contact John Ruger, the USOC Athlete Ombudsman who is completely independent of USADA, or your own personal attorney, for assistance or further information. Mr. Ruger may be reached at One Olympic Plaza, Colorado Springs, CO, 80909, by telephone at (888)-ATHLETE, by fax at (303) 444-6626 or by e-mail at [John.Ruger@usoc.org](mailto:John.Ruger@usoc.org), or at [www.888athlete.org](http://www.888athlete.org).

Sincerely,



Linda M. Barnes

Testing Results Manager

cc: Sean Petty, USA Cycling (w/o encls.)  
Gary Johansen, USOC Deputy General Counsel  
Jim Scherr, USOC Chief Executive Officer  
Christain Varin, UCI Anti-Doping Services (w/o encls.)

Enclosures: Correspondence from UCI to USA Cycling including:  
Doping Control Form  
Laboratory Report of Analysis  
Correspondence from USA Cycling to USADA  
Correspondence from USADA to USA Cycling  
UCI Anti-Doping Rules  
World Anti-Doping Code  
WADA List of Prohibited Substances  
USADA Protocol  
USOC Anti-Doping Policies

UNITED STATES ANTI-DOPING AGENCY  
ACCEPTANCE OF PROVISIONAL SUSPENSION

I, Floyd A. Landis, accept a "provisional suspension" as a result of the finding of a testosterone/epitestosterone ("T/E") ratio greater than 4:1 and a positive Carbon Isotope Ratio ("CIR") analysis in my urine Sample #995474, collected at the Tour de France on July 20, 2006.

I understand and accept that I will not be able to compete in any competitions under the jurisdiction of the Union Cycliste Internationale ("UCI"), USA Cycling, or the United States Olympic Committee ("USOC") or any of these entities' clubs, member associations or affiliates while serving this "provisional suspension."

I understand that the period of the "provisional suspension," beginning on the date I accept this "provisional suspension" and notify USADA of such, will be deducted from any period of ineligibility that I might receive in my case.

I understand and accept that USADA will notify UCI, USA Cycling, and the USOC of my acceptance of the "provisional suspension."

I understand and accept that my acceptance of the "provisional suspension" is purely voluntary and optional. I understand and accept that I am entitled to proceed with my case, to a hearing if necessary, regardless of whether I accept this "provisional suspension."

I understand and accept that I may serve this "provisional suspension" and it may ultimately be determined that no doping offense has occurred by the Panel of the USADA Anti-Doping Review Board or through a hearing.

I understand and accept that I am still subject to testing pending the outcome of this matter.

\_\_\_\_\_  
*Signature of Floyd A. Landis*

\_\_\_\_\_  
*Date*

\_\_\_\_\_  
*Printed Name of Floyd A. Landis*

**Summary by USADA  
Of Laboratory Documents  
For Sample #995474**

<b>Sport:</b>	<b>Cycling</b>
<b>Sample Collection Date:</b>	<b>July 20, 2006</b>
<b>Type of Collection:</b>	<b>Out of Competition</b>
<b>WADA Accredited Laboratory:</b>	<b>University of California at Los Angeles</b>
<b>Substance:</b>	<b>T/E ratio greater than 4:1 CIR Positive</b>
<b>Level of Substance:</b>	<b>T/E ratio approximately 11.4:1</b>

**This summary is based on the laboratory documents provided and is not intended to replace, substitute or in anyway supersede the laboratory documents. This summary is only for general reference purposes.**

## FLOYD LANDIS

## 1. To:

USA CYCLING

To Mr Steve Johnson

By fax: +1 719 785 2028 +1 719 866 4628

## 2. Copy:

## (i) INTERNACIONAL CYCLING UNION (UCI)

To Mr Christian Varin

By fax: +41 24 458 5812

## (ii) USADA

To Mr Travis Tygart

By fax: +1 719 785 2001

## (iii) LNDD - Laboratoire National de Dépistage du Dopage

To Mr Jacques de Ceaurriz

By fax: +33 1 4660 3017

## (iv) PHONAK HEARING SYSTEMS

To Mrs Monika Zuercher

By fax: +41 55 2547011

31st July 2006

Dear Sirs:

I have received on the 26<sup>th</sup> July from my team PHONAK HEARING SYSTEMS copy of a letter sent by UCI that same day concerning my testing positive (T/E) in the Tour de France on the stage of the 20<sup>th</sup> July.

By means of this letter:

- (i) I formally require the B sample analysis, in accordance with articles 191 and following of the Anti-Doping Rules (ADR).
- (ii) I appoint as my legal representatives, Mr Luis Sanz Hernández and Mr José M<sup>a</sup> Buxeda Maisterra, lawyers in Madrid (Spain). I request that any formal communication concerning this issue is sent to the latter (Mr José M<sup>a</sup> Buxeda, MASONS BUXEDA MENCHEN ABOGADOS, calle José Ortega y Gasset, n<sup>o</sup> 29, 28006 - Madrid, T +34-914363325, F +34-914363329, e-mail: [jm.buxeda@mbm-abogados.com](mailto:jm.buxeda@mbm-abogados.com)). According to article 198 ADR, they will attend the opening of sample B.
- (iii) I appoint as the expert that will attend the sample B analysis on my behalf, Dr Douwe de Boer, Department of Clinical Chemistry, University Hospital Maastricht.
- (iv) I require a copy of the complete analysis report for sample A that should be sent to my legal representatives as soon as possible.

- 2 -

- (v) I require from the medical services at the UCI a thorough endocrinological study, to be carried out in a specialised medical center in Europe and the United States.

Yours sincerely,



31/7/06

Floyd Landis

Licence 0020272 - UCI Code USA 19751014



**VIA OVERNIGHT MAIL**

August 30, 2006

Mr. Floyd Landis  
c/o Howard L. Jacobs  
5210 Lewis Road  
Suite 5  
Agoura Hills, CA 91301

Re: UCI File No. 29/06  
Tour de France, July 20, 2006  
Sample #995474

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*Evelyn Ashford*

*Lawrence Brown, Jr., MD, MPH*

*Jean Fourcroy, MD, PhD, MPH*

*Andrew Mecca, Dr PH, MPH*

*Annette Salmeen, DPhil*

Dear Mr. Landis:

On August 3, 2006, the WADA accredited laboratory at Chateauf-Malabry, France ("the Laboratory") analyzed the B sample from your urine specimen #995474 provided at the Tour de France on July 20, 2006. The Laboratory reported that the B sample analysis confirmed the finding of an elevated testosterone/epitestosterone (T/E) ratio at a prohibited level of greater than 4:1. Additionally, the Laboratory performed a Carbon Isotope Ratio ("CIR") analysis on your sample and reported the result positive for exogenous testosterone or its precursors. The World Anti-Doping Agency ("WADA") Prohibited List, adopted by both the USADA Protocol for Olympic Movement Testing ("Protocol") and the Union Cycliste Internationale ("UCI") Anti-Doping Rules, lists testosterone and its precursors as prohibited substances in the class of anabolic androgenic steroids.

We have enclosed for your consideration the following:

1. Correspondence from USADA to you, dated July 27, 2006, containing:
  - a. Cover letter.
  - b. Acceptance of Provisional Suspension Form.
  - c. Summary of Laboratory Documents.
  - d. Correspondence from USA Cycling to USADA dated July 26, 2006, containing:
    - i. Cover Letter.
    - ii. Correspondence from UCI dated July 26, 2006.
    - iii. Doping Control Official Form.
    - iv. The Laboratory Report of Analysis.
  - e. UCI Anti-Doping Rules.
  - f. World Anti-Doping Code.
  - g. The 2006 Prohibited List.
  - h. USADA Protocol for Olympic Movement Testing.

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USADA 1145

2. The Laboratory A documentation package (Bates Labeled USADA 0001 to 0223).
3. The Laboratory B documentation package (Bates Labeled USADA 0224 to 0370).
4. Correspondence from USADA to the Review Board dated August 30, 2006.

At this time, your case is being forwarded to a Panel of the independent Anti-Doping Review Board ("Review Board") for its consideration and recommendation as to whether there is sufficient evidence of doping to proceed to a hearing. Pursuant to the USADA Protocol, which has been provided to you, you have the right to make written submittals to the Review Board for its consideration. Any written submittals must be received by USADA for transmittal to the Review Board by **September 11, 2006**.

The documents submitted by USADA to the Review Board will have your name redacted. USADA will not redact any documents you submit to the Review Board.

Under the applicable rules, doping is strictly forbidden and the finding of a prohibited substance or method in an athlete's body constitutes a doping violation. If it is ultimately determined that a doping violation has occurred, a sanction may be imposed that could result in a two year period of ineligibility and disqualification of any competitive results achieved by you on or subsequent to July 20, 2006, the date your sample was collected. If this case proceeds, USADA will recommend a sanction under the UCI Anti-Doping Rules and the USADA Protocol, both of which adopt the WADA Code. In this event, if you choose to contest the sanction recommended by USADA, you will have the right to request a hearing. If a hearing is held in the regular course, you should anticipate a hearing date before January 1, 2007.

**Also of importance, you are still subject to testing pending the outcome of this matter.**

Additionally, you have the right, at this time, to accept a "provisional suspension." By accepting a "provisional suspension," you will be immediately suspended from competing in all competitions under the jurisdiction of UCI, USA Cycling, and the United States Olympic Committee ("USOC"), or any of these entities' clubs, member associations or affiliates, until your case is deemed not to be a doping offense, you accept a sanction, you fail to contest this matter, or a hearing has been held in this matter. If you choose to accept this "provisional suspension," the time served under the "provisional suspension" will be deducted from any period of ineligibility that you might receive beginning on the date you accept the "provisional suspension" and notify USADA of such acceptance. If you do not to execute

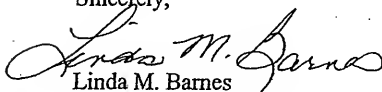
and return this "provisional suspension," any period of ineligibility you might receive will begin on the date of your acceptance of the sanction or on the date of the arbitration hearing panel's decision.

If you execute and return the "provisional suspension," USADA will give notice to the USOC, UCI, and USA Cycling of your acceptance of the "provisional suspension." Your decision to accept this "provisional suspension" is purely optional. You do not have to accept this "provisional suspension" in order to proceed with your case. **If you are willing to accept a "provisional suspension," please inform us in writing immediately, by executing and returning the attached USADA Acceptance of Provisional Suspension Form.**

If you have any questions or need additional information, please feel free to contact me or Travis T. Tygart, USADA's General Counsel. You may also wish to contact your own personal attorney or Mr. John Ruger, the USOC Athlete Ombudsman, who is completely independent of USADA, for assistance. Mr. Ruger may be reached at One Olympic Plaza, Colorado Springs, CO, 80909, by telephone at (888)-ATHLETE, by fax at (303) 444-6626 or by e-mail at [john.ruger@usoc.org](mailto:john.ruger@usoc.org) or at [www.888athlete.org](http://www.888athlete.org).

USADA will not publicly disclose or comment on the specifics of your test results until your case has been resolved. By copy of this letter, USADA is notifying USA Cycling and the USOC of your test results and requests that these organizations not comment publicly concerning this information until disclosed as provided in the USADA Protocol.

Sincerely,

  
Linda M. Barnes  
Testing Results Manager

cc: Sean Petty, USA Cycling (w/o encls.)  
Jeff Gewirtz, USOC General Counsel  
Gary Johansen, USOC Deputy General Counsel  
Jim Scherr, USOC Chief Executive Officer  
Delphine Lautenschlager, UCI Anti-Doping Services (w/o encls.)



UNITED STATES ANTI-DOPING AGENCY  
ACCEPTANCE OF PROVISIONAL SUSPENSION

I, Floyd A. Landis, accept a "provisional suspension" as a result of the finding of a testosterone/epitestosterone ("T/E") ratio greater than 4:1 and a positive Carbon Isotope Ratio ("CIR") analysis in my urine Sample #995474, collected at the Tour de France on July 20, 2006.

I understand and accept that I will not be able to compete in any competitions under the jurisdiction of the Union Cycliste Internationale ("UCI"), USA Cycling, or the United States Olympic Committee ("USOC") or any of these entities' clubs, member associations or affiliates while serving this "provisional suspension."

I understand that the period of the "provisional suspension," beginning on the date I accept this "provisional suspension" and notify USADA of such, will be deducted from any period of ineligibility that I might receive in my case.

I understand and accept that USADA will notify UCI, USA Cycling, and the USOC of my acceptance of the "provisional suspension."

I understand and accept that my acceptance of the "provisional suspension" is purely voluntary and optional. I understand and accept that I am entitled to proceed with my case, to a hearing if necessary, regardless of whether I accept this "provisional suspension."

I understand and accept that I may serve this "provisional suspension" and it may ultimately be determined that no doping offense has occurred by the Panel of the USADA Anti-Doping Review Board or through a hearing.

I understand and accept that I am still subject to testing pending the outcome of this matter.

\_\_\_\_\_  
*Signature of Floyd A. Landis*

\_\_\_\_\_  
*Date*

\_\_\_\_\_  
*Printed Name of Floyd A. Landis*



VIA OVERNIGHT MAIL

September 19, 2006

Mr. Floyd Landis  
c/o Howard L. Jacobs  
5210 Lewis Road  
Suite 5  
Agoura Hills, CA 91301

Re: UCI File No. 29/06  
Tour de France, July 20, 2006  
Sample #995474

*Board of Directors*

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*Evelyn Ashford*

*Lawrence Brown, Jr., MD, MPH*

*Jean Fourcay, MD, PhD, MPH*

*Andrew Mecca, Dr PH, MPH*

*Annette Solmeier, DPhil*

Dear Mr. Landis:

The Panel of the United States Anti-Doping Agency ("USADA") Anti-Doping Review Board ("Review Board") met concerning your positive sample #995474 provided on July 20, 2006, at the Tour de France. The Review Board determined there was sufficient evidence of a doping violation and recommended that the adjudication process proceed as set forth pursuant to the USADA Protocol for Olympic Movement Testing ("Protocol") and the Union Cycliste Internationale ("UCI") Anti-Doping Rules, both of which adopt the World Anti-Doping Agency ("WADA") Code. A copy of the Panel's recommendation is enclosed.

Therefore, at this time, reserving all rights to amend this charge, USADA charges you with a doping violation for testing positive for exogenous testosterone or its precursors as conclusively established by Carbon Isotope Ratio ("CIR") analysis and further corroborated by an elevated testosterone to epitestosterone ("T/E") ratio in this sample, which could only be compatible with exogenous administration. Under the USADA Protocol and the UCI Anti-Doping Rules, both of which incorporate the WADA Code, doping is strictly forbidden and is an offense.

USADA applies the sanctions found in the applicable rules and United States Olympic Committee ("USOC") Anti-Doping Policies. Pursuant to the USADA Protocol, the UCI Anti-Doping Rules, and the USOC Anti-Doping Policies, all of which have previously been provided to you, you are subject to the following sanction for a first doping violation:

- A two (2) year period of ineligibility as described by the WADA Code, beginning on the day you accept this sanction, fail to contest this sanction, or the date of the hearing decision in this matter; and,

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[usada@usantidoping.org](mailto:usada@usantidoping.org) ■ [www.usantidoping.org](http://www.usantidoping.org)

- Disqualification of all competitive results obtained on or subsequent to July 20, 2006, the date your sample was collected, including forfeiture of any medals, points and prizes; and,
- Ineligibility for a period of two (2) years beginning on the day you accept this sanction, fail to contest this sanction or the date of the hearing decision in this matter, from participating or coaching in U.S. Olympic, Pan American Games or Paralympic Games Trials, being a member of any U.S. Olympic, Pan American Games or Paralympic Team and having access to the training facilities of the USOC Training Centers or other programs and activities of the USOC including, but not limited to benefits, grants, awards or employment as set forth in Section 6 of the USOC Anti-Doping Policies and further defined by Annex C therein.

As required in every doping case under the USADA Protocol, your doping violation and the resulting sanction will be publicly announced.

If you are willing to accept this sanction, please inform us in writing by September 29, 2006, by executing and returning the attached USADA Acceptance of Sanction Form. Please return it by fax to 719-785-2028.

If you choose to contest the sanction proposed by USADA, you have the right to request a hearing. As described in the USADA Protocol, you must inform us in writing by September 29, 2006, if you elect to proceed to a hearing before the American Arbitration Association ("AAA") arbitrator(s) selected from a pool of the North American Court of Arbitration for Sport ("CAS") Arbitrators as described in 10(a) and 10(b) of the Protocol. I have enclosed a copy of the Protocol for your reference. If you fail to notify USADA in writing of your intention to contest this sanction before September 29, 2006, or have not requested a five day extension as described in 10(a) of the Protocol, the sanction will go into effect on that date and USADA will make a public announcement concerning your doping violation and its consequences.

Also of importance, you are still subject to testing pending the outcome of this matter.

Additionally, you have the right, at this time, to accept a "provisional suspension." By accepting a "provisional suspension," you will be immediately suspended from competing in all competitions under the jurisdiction of UCI, USA Cycling, and the United States Olympic Committee ("USOC"), or any of these entities' clubs, member associations or affiliates, until your case is deemed not to be a doping offense, you accept a sanction, you fail to contest this matter, or a hearing has been held in this matter. If you choose to accept this "provisional suspension," the time served under the

"provisional suspension" will be deducted from any period of ineligibility that you might receive beginning on the date you accept the "provisional suspension" and notify USADA of such acceptance. If you do not execute and return this "provisional suspension," any period of ineligibility you might receive will begin on the date of your acceptance of the sanction or on the date of the arbitration hearing panel's decision.

If you execute and return the "provisional suspension," USADA will give notice to the USOC, UCI, and USA Cycling of your acceptance of the "provisional suspension." Your decision to accept this "provisional suspension" is purely optional. You do not have to accept this "provisional suspension" in order to proceed with your case. If you are willing to accept a "provisional suspension," please inform us in writing immediately, by executing and returning the attached USADA Acceptance of Provisional Suspension Form.

If you have any questions or need additional information, please feel free to contact me. As advised in previous correspondence concerning this matter, you may also contact John Ruger, the USOC Athlete Ombudsman who is completely independent of USADA, at One Olympic Plaza, Colorado Springs, CO, 80909, by telephone at (888)-ATHLETE, by fax at (303) 444-6626 or by e-mail at [www.888athlete.org](http://www.888athlete.org).

USADA will not publicly disclose or comment on the specifics of your test results until your case has been resolved. By copy of this letter, USADA is notifying the UCI, WADA, USA Cycling and the USOC of your test results and requests that these organizations not comment publicly concerning this information until disclosed as provided in the USADA Protocol

Sincerely,

  
Travis T. Tygart  
General Counsel

cc: Sean Petty, USA Cycling  
Jeff Gewirtz, USOC General Counsel  
Gary Johansen, USOC Associate General Counsel  
Jim Scherr, USOC Chief Executive Officer  
Rune Anderson, WADA  
Delphine Lautenschlager, UCI

UNITED STATES ANTI-DOPING AGENCY  
ACCEPTANCE OF PROVISIONAL SUSPENSION

I, Floyd A. Landis, accept a "provisional suspension" as a result of the finding of exogenous testosterone or its precursors as conclusively established by Carbon Isotope Ratio ("CIR") analysis and further corroborated by an elevated testosterone to epitestosterone ("T/E") ratio in my urine Sample #995474, collected at the Tour de France on July 20, 2006.

I understand and accept that I will not be able to compete in any competitions under the jurisdiction of the Union Cycliste Internationale ("UCI"), USA Cycling, or the United States Olympic Committee ("USOC") or any of these entities' clubs, member associations or affiliates while serving this "provisional suspension."

I understand that the period of the "provisional suspension," beginning on the date I accept this "provisional suspension" and notify USADA of such, will be deducted from any period of ineligibility that I might receive in my case.

I understand and accept that USADA will notify UCI, USA Cycling, and the USOC of my acceptance of the "provisional suspension."

I understand and accept that my acceptance of the "provisional suspension" is purely voluntary and optional. I understand and accept that I am entitled to proceed with my case, to a hearing if necessary, regardless of whether I accept this "provisional suspension."

I understand and accept that I may serve this "provisional suspension" and it may ultimately be determined that no doping offense has occurred by the Panel of the USADA Anti-Doping Review Board or through a hearing.

I understand and accept that I am still subject to testing pending the outcome of this matter.

Signature of Floyd A. Landis

Date

Printed Name of Floyd A. Landis

## UNITED STATES ANTI-DOPING AGENCY

### ACCEPTANCE OF SANCTION

I, Floyd Landis, accept the following sanction as a result of my first doping offense arising from my urine Sample #995474 provided on July 20, 2006, at the Tour de France, which tested positive for the prohibited substance exogenous testosterone or its precursors as conclusively established by Carbon Isotope Ratio ("CIR") analysis and further corroborated by an elevated testosterone to epitestosterone ("T/E") ratio in my sample, which could only be compatible with exogenous administration. I acknowledge that I have violated applicable rules, including the USADA Protocol for Olympic Movement Testing ("Protocol") and the UCI Anti-Doping Rules, both of which have adopted the World Anti-Doping Code ("WADA Code"), and I accept the following:

- A two (2) year period of ineligibility under Article 10 of the WADA Code beginning on the day I accept this sanction; and,
- Pursuant to Article 10 of the WADA Code, disqualification of all competitive results obtained on or subsequent to July 20, 2006, the date my sample was collected, including forfeiture of any medals, points and prizes; and,
- Ineligibility for a period of two (2) years beginning on the day I accept this sanction, from participating or coaching in U.S. Olympic, Pan American Games or Paralympic Games Trials, being a member of any U.S. Olympic, Pan American Games or Paralympic Team and having access to the training facilities of the USOC Training Centers or other programs and activities of the USOC including, but not limited to, grants, awards or employment as set forth in Section 6 of the USOC Anti-Doping Policies and further defined by Annex C therein; and,
- Suspension of USOC benefits pursuant to Annex C of the USOC Anti-Doping Policies.

I do not contest the above sanction determined by USADA under the UCI Anti-Doping Rules, since the prohibited substance testosterone or its precursors were found in my urine sample and I have agreed to the violation. I understand that USADA will communicate my acceptance to the UCI and USA Cycling who will impose this sanction. I voluntarily, knowingly, and intelligently waive any and all rights to contest the results of the UCLA Laboratory or the sanction determined by USADA.

I also understand and accept that under the USADA Protocol, my doping violation and the resulting sanction will be publicly announced.

I understand and accept that entities other than the USOC, UCI and USA Cycling will give effect to this sanction including, but not limited to, the National Collegiate Athletic Association and National Association of Intercollegiate Athletics, if applicable. I understand and accept that it is my obligation to investigate the effect of this sanction on me by other entities.

Also, in accordance with the USADA Protocol and the USOC Anti-Doping Policies, in order to regain eligibility, I must comply with all requirements of the UCI and the USADA Out of Competition testing program and the requirements of other applicable reinstatement testing rules during the period of my suspension, including the requirement to update USADA of my location so that I may be tested. I understand that if I retire and/or fail to comply with these requirements, additional time will be added to my period of ineligibility pursuant to Article 10.10 of the WADA Code, which is incorporated into the applicable rules noted above. In order to regain eligibility, I accept that I must also comply with the requirements of the UCI Anti-Doping Rules, if any.

\_\_\_\_\_  
*Signature of Floyd Landis*

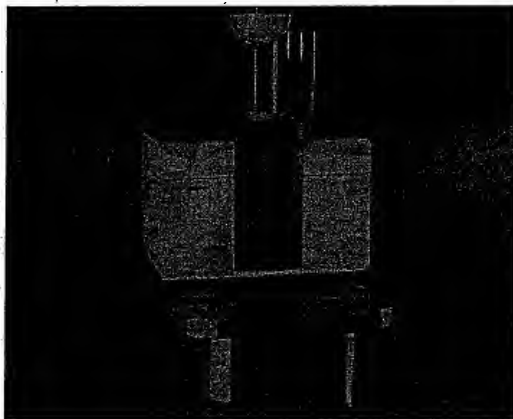
\_\_\_\_\_  
*Date*

\_\_\_\_\_  
*Printed Name of Floyd Landis*

**TAB 48**

IsoPrime EA User Manual  
Code No 6666588  
Issue 1a

## Section 5



## User Interface



**Trap bake temperature**

Not used on IsoPrime.

**Maximum cooling time**

Not used on IsoPrime.

**Magnet Constant**

General Mass Spectrometer equation used when HT peak jumping and is updated when the peak is identified.

**HT Settle Time**

This is the time allowed after a HT peak jump for the system to settle prior to data being measured.

**Hall Probe Enabled**

This location stores whether the Hall Probe has been enabled.

**Use Mass Cal With Hall Probe**

This location stores whether the mass calibration used the Hall Probe.

**Reference bellows maximum**

Not used on IsoPrime.

**Sample bellows maximum**

Not used on IsoPrime.

**Amplifier Zero Channel 0**

The location of the main analyser Low 1 collector amplifier zero is stored.

**Amplifier Zero Channel 1**

The location of the main analyser Axial collector amplifier zero is stored.

**Amplifier Zero Channel 2**

The location of the main analyser High collector amplifier zero is stored.

**Amplifier Zero Channel 3**

The location of the main analyser Low 2 collector amplifier zero is stored.

**Amplifier Zero Channel 4**

Not used on IsoPrime

**Amplifier Zero Channel 5**

Not used on IsoPrime

**Amplifier Zero Channel 6**

Not used on IsoPrime

**Amplifier Zero Channel 7**

Not used on IsoPrime

**Amplifier Zero Channel 8**

Not used on IsoPrime.

**TAB 49**

**Kemp, Stuart**

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**From:** Kemp, Stuart  
**Sent:** Wednesday, July 12, 2006 9:03 AM  
**To:** 'Varin Christian - UCI'  
**Cc:** 'Querzola Pascale - UCI'  
**Subject:** RE: WADA OOC Testing  
**Attachments:** Landis Attempt.pdf

Good day Christian,

Regarding the attempt on Floyd Landis, the attempt made was not consistent with the whereabouts information provided by the athlete and therefore not considered a possible missed test. I have attached the related documentation for your reference, as you will note the DCO made an attempt the day after Mr. Landis indicated he would be leaving home for the TdF.

Kind regards,

Stuart

**Stuart KEMP**

Testing Manager  
Standards and Harmonization  
World Anti-Doping Agency  
Agence Mondiale Antidopage  
Tel: +1 514 904 8836  
Fax: +1 514 904 2266  
E-mail: [stuart.kemp@wada-ama.org](mailto:stuart.kemp@wada-ama.org)  
Web: <http://www.wada-ama.org>

---

**From:** Varin Christian - UCI [mailto:[christian.varin@uci.ch](mailto:christian.varin@uci.ch)]  
**Sent:** Wednesday, July 12, 2006 3:38 AM  
**To:** Kemp, Stuart  
**Cc:** Querzola Pascale - UCI  
**Subject:** RE: WADA OOC Testing  
**Importance:** High

Dear Stuart,

I refer to your email sent last 7<sup>th</sup> July 2006

I did not find any information regarding an unsuccessful mission for Floyd Landis?

Could you please email me the information?

Thank you for your help on this matter!

Yours sincerely,

Christian Varin  
Manager Antidopage / Antidoping Manager  
Union Cycliste Internationale  
1860 Aigle

Tél: +41-24.468.58.11  
Fax: +41-24.468.58.12  
[www.uci.ch](http://www.uci.ch)

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**IMPORTANT NOTICE :**

This message contains confidential information and is intended only for the individual named herein. If you are not the herein named addressee you should not disseminate, distribute copy or otherwise make use of this e-mail. Please notify the sender immediately by e-mail if you have received this e-mail by mistake, and delete this e-mail from your system.

---

**From:** Kemp, Stuart [mailto:Stuart.Kemp@wada-ama.org]  
**Sent:** vendredi, 7. juillet 2006 17:03  
**To:** Varin Christian - UCI  
**Subject:** WADA OOC Testing

Good day Christian,

Further to the two recent unsuccessful mission reports that I've faxed you this week, I wanted to advise you that an attempt was made to test Floyd Landis on June 26<sup>th</sup> in Gerona. Unfortunately, the USADA whereabouts information used was not sufficient to successfully locate the athlete. Should you require any further information on this attempt, please do not hesitate in contacting me.

Thank you for your cooperation with this particular mission and have a pleasant weekend.

Best regards,

Stuart

**Stuart KEMP**  
Testing Manager  
Standards and Harmonization  
World Anti-Doping Agency  
Agence Mondiale Antidopage  
**E-mail:** [Stuart.Kemp@wada-ama.org](mailto:Stuart.Kemp@wada-ama.org)  
**Web:** <http://www.wada-ama.org>



ADAMS Mission Order #: M-232700

**SAMPLE COLLECTION ORDER-1**

<b>Date Issued</b>	<b>Primary DCO</b>	<b>Status</b>
June 22, 2006		Issued
<b>Issued By/Test Authority</b>	<b>Sample Collection Authority</b>	<b>Result Management Authority</b>
WADA World Anti-Doping Agency 800 Place Victoria Suite 1700 Montreal Quebec CANADA H4Z1B7	IDTM International Doping Tests and Manag Stockholmsvägen 18 Lidingö Stockholm län [SE-01] SWEDEN 18133	UCI International Cycling Union Aigle Vaud (fr) SWITZERLAND 1860
Rune Andersen Fax: +1 514 904 8823	Veronika Lyckow (Operations) +46 8 555 109 00	Christian Varin 0041 24 468 58 11

<b>Name of Competition/Training Session</b>	<b>ADO Reference #</b>	<b>PSN Type</b>
UCI Individual athlete, Spain June		<input type="checkbox"/> In Competition <input checked="" type="checkbox"/> Out of Competition
<b>Country of Mission</b>	<b>Sample Collection Dates</b>	
SPAIN	From: June 22, 2006	To: June 27, 2006
<b>Region of Mission</b>	<b>Description of Mission</b>	
	UCI Individual athlete, Spain June	
<b>City of Mission</b>		

<b>Send Notification of Results to:</b>	
WADA World Anti-Doping Agency 800 Place Victoria Suite 1700 Montreal Quebec CANADA H4Z1B7	UCI International Cycling Union Aigle Vaud (fr) SWITZERLAND 1860
Rune Andersen Fax: +1 514 904 8823	Christian Varin 0041 24 468 58 11

<b>Status Instruction/Additional Information</b>					
Athlete is US athlete who lives in Spain: 7-2-1 Riera Buganto, Girona, Spain, Athlete likely to leave for Tour de France by June 27th - please make more than one attempt to test if possible.					
<b>Selection Policy Used/Details of Selection</b>					
Please continue to collect samples until required specific gravity is met. Testing must be no-advance notice.					
<b>Testing Team/Participants</b>					
Role	Last Name	First Name	Role	Last Name	First Name

WADA0003

**Confidential!**  
**UNSUCCESSFUL ATTEMPT FORM**  
 Please write legibly and in CAPITAL letters

Name of person to be tested <b>FLOYD LANDIS</b>	Gender <input checked="" type="checkbox"/> M <input type="checkbox"/> F	Nationality <b>USA</b>
Name of organisation requesting the test <b>WADA / UCI</b>	Sport/Event <b>CYCLING</b>	
Name of DCO <b>NURIA DE PINO</b>	Type of test <input checked="" type="checkbox"/> Out-of-Competition <input type="checkbox"/> Competition	<input checked="" type="checkbox"/> Urine test <input type="checkbox"/> Blood test
Name of Assistant <b>JOSE A. MORENO</b>	IDTM's Ref. No. <b>M-232700</b>	DCO license No. <b>455</b>
MISSION PLANNED BASED ON WHEREABOUTS PROVIDED FOR		
Year: <b>2006</b> <input checked="" type="checkbox"/> Quarter Last update to Whereabouts provided by IDTM (date) <b>26/06/06</b>		

**Attempt Information**

<b>LOCATION/S VISITED</b>			
<b>CITY:</b> <b>GIRONA</b>	<b>STATE:</b>	<b>COUNTRY:</b> <b>SPAIN</b>	
<b>I HAVE VISITED</b>	<b>Date (DD/MM/YY)</b>	<b>Arrival Time</b>	<b>Departure Time</b>
<input type="checkbox"/> Training Place 1	____/____/____	____ AM <input type="checkbox"/> PM	____ AM <input type="checkbox"/> PM
	____/____/____	____ AM <input type="checkbox"/> PM	____ AM <input type="checkbox"/> PM
I have talked to: <input type="checkbox"/> Contact Person <input type="checkbox"/> Coach <input type="checkbox"/> Other (parents etc.)			
Address & Telephone of above person/s: _____			
Information provided: _____			
<input type="checkbox"/> Training Place 2 (if applicable)	____/____/____	____ AM <input type="checkbox"/> PM	____ AM <input type="checkbox"/> PM
	____/____/____	____ AM <input type="checkbox"/> PM	____ AM <input type="checkbox"/> PM
I have talked to: <input type="checkbox"/> Contact Person <input type="checkbox"/> Coach <input type="checkbox"/> Other (parents etc.)			
Address & Telephone of above person/s: _____			
Information provided: _____			
<input checked="" type="checkbox"/> Current Address or Temporary Address	<b>26/06/06</b>	<b>18:00</b> <input type="checkbox"/> AM <input checked="" type="checkbox"/> PM	<b>19:00</b> <input type="checkbox"/> AM <input checked="" type="checkbox"/> PM
	____/____/____	____ AM <input type="checkbox"/> PM	____ AM <input type="checkbox"/> PM
I have talked to: <input type="checkbox"/> Contact Person <input type="checkbox"/> Coach <input checked="" type="checkbox"/> Other (parents etc.)			
Address & Telephone of above person/s: <b>FRIEND</b>			
Information provided: <b>HE TOLD US THAT HE WAS NOT THERE, AND THEN I ASKED HIM IF HE KNEW WHEN HE IS GOING TO COME, AND HE SAID THAT HE DIDN'T KNOW BECAUSE LANDIS WAS TRAVELLING</b>			
<input type="checkbox"/> I have contacted the following person by telephone (if applicable): _____			
Information provided: _____			
<b>Comments: (For additional comments, use a separate sheet of paper)</b>			
<b>WE ARRIVED TO THE ADDRESS GIVEN IN THE WADA AND RANG ON THE PHONE-NO. A FRIEND OF HIS ANSWERED AND SAID US THAT LANDIS WAS NOT THERE AND THAT HE WAS TRAVELLING AND HE DID NOT KNOW WHEN HE IS GOING TO RETURN. EVEN THOUGH THE INFORMATION WE WAIT IN FRONT OF HIS HOUSE FOR ONE HOUR.</b>			

**Confirmation of IDCO**

☒ I am aware that by reporting that the athlete is unavailable he/she can be punished for violating the rules of the organisation.  
 I declare that the information I have given above is true  
 Signature of Doping Control Officer

Signature of Doping Control Assistant

WADA 0004

## MISSION SUMMARY

First Name: Nuria	Last Name: del Pino
Sport/Federation: WADA/UCI	Reference No: M-232700
Date/s of Mission: 26/06/06	City/s & Country: Girona (Spain)
No. urine samples collected: 0	No of Blood samples collected:
I've been working on this mission as a:	DCO

### Mission introduction comments:

We arrived to the address given in the WA and rang on the phone-door, a friend of him answer and said us that Landis was not there and that he was travelling and he did not know when he is going to return. Even though the information provided from his friend we wait in front of his house for one hour in case he arrives.

### Name/s unavailable athlete/s:

Floyd Landis

### DCO Signature:

Nuria del Pino Blasco

Date: 26/06/06

REC'D JUN 01 2006

ENT'D JUN 01 2006



**ATHLETE LOCATION FORM**  
**QUARTERLY UPDATE: July-September**  
**DUE AT USADA: June 1st, 2006**  
**FAX 719-785-2099**

If you have an email address on file with USADA you can submit this form online at: [www.usantidoping.org](http://www.usantidoping.org)  
 Please type or print legibly. **Do not use pencil.**

**Athlete Information**

(THIS INFORMATION IS REQUIRED FOR YOUR FORM TO BE CONSIDERED COMPLETE.)

**Residence** Providing detailed contact information is to your benefit and can help minimize the likelihood of you being declared "Unavailable".

NAME: Landis Floyd  
 GENDER: ☒ MALE ☐ FEMALE

DATE OF BIRTH: 10 / 14 / 75  
month day year

PHYSICAL ADDRESS: (Where you will reside this quarter. No P.O. Boxes.)  
23356 Bishop Rd.

MAILING ADDRESS: (If different from physical address)

Murmeta Ca. 92542  
city state zip code

City State zip code country

EMAIL ADDRESS: Floydlandis@hotmail.com

PRIMARY CONTACT PERSON: Michael Lutherford  
(Provide your Email Address in order to be eligible to submit electronic Athlete Location Forms. Changes of Name Forms and emailed Updates)

303.513.1304  
Telephone number of contact

SPORT: cycling

DISCIPLINE:

PARALYMPIC ☐ YES PARALYMPIC CLASSIFICATION:

**Regular Quarterly Schedule** (See instructions for more detail on how to complete this section.)

This form is designed for use by a large number of Athletes. We realize some schedules are more complex than others and encourage you to attach additional information on separate sheets if necessary. This is your chance to control when and where you can be contacted for OOC testing.

**Primary Training Location**

FACILITY NAME:

FACILITY ADDRESS:

PRIMARY TRAINING LOCATION SCHEDULE: (Please indicate specific times: i.e. 9:00 a.m. - 2:00 p.m.)

Day	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
A.M.							
P.M.							

**Secondary Training/Alternate Location**

FACILITY NAME:

FACILITY ADDRESS:

SECONDARY TRAINING/ALTERNATE LOCATION SCHEDULE: (Please indicate specific times: i.e. 9:00 a.m. - 2:00 p.m.)

Day	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
A.M.							
P.M.							

**Other Regular Activities** (for additional Other Regular Activities, please attach a separate sheet of paper)

Please provide address for activities for which you check "Yes" to being tested. Please provide specific times (i.e. 9:00 a.m. - 2:00 p.m.).

ACTIVITY 1:

ADDRESS:

ACTIVITY 2:

ADDRESS:

MAY WE TEST DURING THIS ACTIVITY?

☐ YES

☐ NO

MAY WE TEST DURING THIS ACTIVITY?

☐ YES

☐ NO

DAY SUN MON TUE WED THU FRI SAT

DAY SUN MON TUE WED THU FRI SAT

06



EXCEPTIONS TO QUARTERLY SCHEDULE (See instructions for more detail on how to complete this section.)

NAME

Lands

TEMPORARY ADDRESSES (Please attach additional sheets as necessary - be sure to write your name on any additional sheets submitted.)

Pho

Temporary Residence Address

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

Competition Schedule:

Competition

Location

Country

FRANCE

Competition Dates

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Competition Dates

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

Competition Dates

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

ZIP CODE

Country (if other than U.S.)

Temporary Training Address (if applicable)

STARTING DATE TO ENDING DATE

TRIP DATES (Please list each day of travel)

CITY

STATE

I acknowledge that this form, and the information submitted, may be shared with the World Anti-Doping Agency on the condition that the information is to be used for doping control purposes only.

ATHLETE SIGNATURE:

Amberlandi

(Wife) 5-31-09

SIGNATURE IS REQUIRED FOR FORM TO BE CONSIDERED COMPLETE.

**Clark Kelley**

---

**From:** Floyd Landis [floydlandis@hotmail.com]  
**Sent:** Sunday, April 30, 2006 9:40 PM  
**To:** USADA Forms Update  
**Subject:** update [ME-060430-469434]

Hello again

Sorry this is a day late, but I had to leave California a week early and am now in Spain until further notice. Address: 7-2-1 Riera Buganto, Gerona, Spain.

Thanks  
Floyd Landis



## Analyses

### Analysis (1)

**Sample Type**

Urine

## Analyses

EPO

## Lab

LAB Madrid  
Laboratorio de Control del Dopaje  
del Consejo Superior de Deportes c/  
El Greco. s/n  
Madrid  
SPAIN 28040

**Dr. Augustin Francisco Rodriguez**  
+34 91 589 6889/88

### Analysis (2)

### Sample Type

## Analyses

Lab

### Notes/Analyses Instructions

### Notes/Analyses/Instructions

## Athlete List

## Companying Documentation

### Letter of Authority

WADA Letter of Authority

## Athlete Guide

### Athlete Feedback Form

### Ranking List

## Whereabouts Information

## Competition Calendar

## DCO Instructions

Other (please specify)

### Hotel Map and Address

## UNSUCCESSFUL ATTEMPT FORM

Please write legibly and in CAPITAL letters.

Name of person to be tested <b>FLOYD LANDIS</b>	Gender <input checked="" type="checkbox"/> M <input type="checkbox"/> F	Nationality <b>USA</b>
Name of organisation requesting the test <b>WADA / UCI</b>	Sport/Event <b>CYCLING</b>	
Name of DCO <b>NURIA DEL PINO</b>	Type of test <input checked="" type="checkbox"/> Out-of-Competition <input type="checkbox"/> Competition	<input checked="" type="checkbox"/> Urine test <input type="checkbox"/> Blood test
Name of Assistant <b>JOSE A. MORENO</b>	IDTM's Ref. No. <b>H-232700</b>	DCO license No. <b>455</b>
MISSION PLANNED BASED ON WHEREABOUTS PROVIDED FOR		
Year: <b>2006</b> <input checked="" type="checkbox"/> Quarter Last update to Whereabouts provided by IDTM (date) <b>26/06/06</b>		

## Attempt Information

## LOCATION/S VISITED

CITY: **GIRONA**

STATE:

COUNTRY: **SPAIN**

## I HAVE VISITED

Date (DD/MM/YY)

Arrival Time

Departure Time

☐ Training Place 1

\_\_\_\_/\_\_\_\_/\_\_\_\_

\_\_\_\_ AM ☐ PM\_\_\_\_ AM ☐ PMI have talked to: ☐ Contact Person ☐ Coach ☐ Other (parents etc.)

Address &amp; Telephone of above person/s: \_\_\_\_\_

Information provided: \_\_\_\_\_

☐ Training Place 2  
(if applicable)

\_\_\_\_/\_\_\_\_/\_\_\_\_

\_\_\_\_ AM ☐ PM\_\_\_\_ AM ☐ PMI have talked to: ☐ Contact Person ☐ Coach ☐ Other (parents etc.)

Address &amp; Telephone of above person/s: \_\_\_\_\_

Information provided: \_\_\_\_\_

☒ Current Address  
or Temporary Address**26/06/06****18:00** AM ☒ PM**19:00** AM ☒ PMI have talked to: ☐ Contact Person ☐ Coach ☒ Other (parents etc.)Address & Telephone of above person/s: **FRIEND**Information provided: **HE TOLD US THAT HE WAS NOT THERE, AND THEN I ASKED HIM IF HE KNEW WHEN HE IS GOING TO COME, AND HE SAID THAT HE DIDN'T KNOW BECAUSE LANDIS WAS TRAVELLING**☐ I have contacted the following person by telephone (if applicable): \_\_\_\_\_

Information provided: \_\_\_\_\_

Comments: (For additional comments, use a separate sheet of paper)

**WE ARRIVED TO THE ADDRESS GIVEN IN THE WADA AND RANG ON THE PHONE-DOOR. A FRIEND OF HIS ANSWERED AND SAID US THAT LANDIS WAS NOT THERE AND THAT HE WAS TRAVELLING AND HE DID NOT KNOW WHEN HE IS GOING TO RETURN. EVEN THOUGH THE INFORMATION WE WAIT IN FRONT OF HIS HOUSE FOR ONE HOUR.**

## Confirmation of IDCO

☒ I am aware that by reporting that the athlete is unavailable he/she can be punished for violating the rules of the organisation.

I declare that the information I have given above is true

Signature of Doping Control Officer

Signature of Doping Control Assistant

WADA0010

## WADA Technical Document – TD2004EAAS

Document Number:	TD2004EAAS	Version Number:	1.0
Written by:	WADA Laboratory Committee	Approved by:	WADA Executive Committee
Date:	30 May, 2004	Effective Date:	13 August, 2004

## REPORTING AND EVALUATION GUIDANCE FOR TESTOSTERONE, EPITESTOSTERONE, T/E RATIO AND OTHER ENDOGENOUS STEROIDS

### 1. Introduction:

This guide has been prepared to ensure that Laboratories can report, in a uniform way, the presence of abnormal profiles of urinary steroids resulting from the administration of testosterone or its precursors, androstenediol, androstenedione, dehydroepiandrosterone (DHEA) or a testosterone metabolite, dihydrotestosterone or a masking agent, epitestosterone. It also provides guidance to the Testing Authority on how to conduct the evaluation of *Adverse Analytical Findings* reported by the Laboratories.

It is proven that administration of these steroids alters one or more of the parameters of the urinary steroid profile. Elevated levels of urinary metabolites, which are part of the "steroid profile", e.g. testosterone, epitestosterone, dihydrotestosterone, androsterone, etiocholanolone, DHEA as well as other specific metabolites are not consistent with normal endogenous production and result from the intake of these steroids. Increased ratios of specific pairs of steroid metabolites are also indicative of the administration of these endogenous steroids.

It is emphasized that the following requirements shall be applied by all Laboratories in their routine practice.

### 2. Specific requirements for GC/MS measurement of T/E value, concentration of testosterone, concentration of epitestosterone:

The T/E value is given by the peak area or peak height ratio of testosterone and epitestosterone (equivalent to the glucuronide) obtained by measuring the ion at m/z 432 by GC/MS analysis in a Single Ion Monitoring mode (SIM). The T/E value is usually measurable regardless of the concentration of both steroids. Whether measured from the Screening Procedure or the Confirmation Procedure, it must be corrected using an appropriate standard (e.g. calibration curve, quality control sample(s) or authentic standard solutions of both testosterone and epitestosterone). The concentration of testosterone and epitestosterone (equivalent to the glucuronide) should be estimated but should not be used to determine the T/E value. In the case of high T/E values, the concentration of epitestosterone is frequently low and it may not always be possible to measure epitestosterone precisely. In such cases, only the concentration of testosterone (equivalent to the glucuronide) is to be determined.

The Screening Procedure which is normally conducted on a single aliquot shall be carried out including, together in the same batch, a control sample where the T/E value, concentrations of testosterone and epitestosterone are known.

Reference ranges of the various parameters of the urinary steroid profile have been described for populations of both males and females. It should be borne in mind that there is significant

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variation between individuals. A normal level for one individual may in another be elevated and be consistent with doping. The Laboratory will adapt its testing procedures to the *Sample* tested; for example, female or male, Asian or Caucasian (when the information is provided). The concentration of urinary steroids such as testosterone and epitestosterone varies greatly between individuals and also depends upon the specific gravity of the urine *Sample*; only values corrected for a specific gravity value of 1.020 can be compared.

It is recommended that a urine *Sample* in which any one of the following criteria is met during the Screening Procedure, be routinely submitted to the IRMS analysis:

- i) T/E value equal or greater than 4;
- ii) concentration of testosterone or epitestosterone (equivalent to the glucuronide) greater than 200 ng/mL<sup>1</sup>;
- iii) concentration of androsterone or etiocholanolone (equivalent to the glucuronide) greater than 10,000 ng/mL<sup>1</sup>;
- iv) concentration of DHEA (equivalent to the glucuronide) greater than 100 ng/mL<sup>1</sup>.

It is recognised that other parameters may justify a need for IRMS study and the reason should be documented.

Any result that will be used to support an *Adverse Analytical Finding* shall be confirmed and quantified.

Confirmation of elevated T/E values, concentration of testosterone, epitestosterone or any other steroid metabolite under consideration is to be performed in triplicate. The confirmation of the identity of any steroid reported with abnormal properties must be made (refer to technical document TD2003IDCR). Appropriate calibration (e.g. calibration curve, deuterated standards, quality control samples) is to be included in the protocol of the Confirmation Procedure.

Confirmed elevated concentration of steroids will be reported as such together with the value adjusted for the specific gravity of the urine *Sample* using the following formula:

$$\text{Concentration}_{1.020} \text{ ng/mL} = (1.020 - 1) / (\text{Specific gravity of the Sample} - 1) \cdot \text{Concentration measured ng/mL}$$

The urine *Sample* is not collected under sterile conditions, and where the circumstances are favourable, the microbes present in the *Sample* can cause changes to the profile of the urinary steroids. Initially there is cleavage of the glucuronides and sulfates followed by modifications of the steroids' structure by oxido-reductive reactions. To report an *Adverse Analytical Finding* of an elevated T/E value, testosterone or epitestosterone concentration or any other endogenous steroid parameters, the concentration of free testosterone and/or epitestosterone in the specimen is not to exceed 5% of the respective glucuroconjugates. Elevated amounts of 5 $\alpha$ - and 5 $\beta$ -androstan-3,17-dione in the free form also indicate microbial degradation.

<sup>1</sup> Concentrations adjusted for a specific gravity value of 1.020

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### 3. Isotope ratio mass spectrometry:

When a parameter of the steroid profile indicates a need to further study, its  $^{13}\text{C}/^{12}\text{C}$  value expressed in delta units per mil ( $\delta\text{‰}$ ) or that of its metabolites will be measured and compared to that of urinary reference steroids within the sample not affected by administration. Depending upon the nature of the endogenous steroid suspected to have been administered, the metabolites analysed could be testosterone, epitestosterone, androsterone, etiocholanolone, the androstanediols, DHEA, or other relevant metabolites while the urinary reference steroid usually analysed by the Laboratories is one of, pregnanediol, pregnanetriol, cholesterol, 11-hydroxyandrosterone or 11-ketoetiocholanolone. The instrumentation should be calibrated with an appropriate Reference Material.

The results will be reported as consistent with the administration of a steroid when the  $^{13}\text{C}/^{12}\text{C}$  value measured for the metabolite(s) differs significantly i.e. by 3 delta units or more from that of the urinary reference steroid chosen. In some *Samples*, the measure of the  $^{13}\text{C}/^{12}\text{C}$  value of the urinary reference steroid(s) may not be possible due to their low concentration. The results of such analyses will be reported as “inconclusive” unless the ratio measured for the metabolite(s) is below -28‰ based on non-derivatised steroid.

### 4. Reviewing and evaluating test results:

The following actions should be requested by the Testing Authority in agreement with the Laboratory:

- Isotopic ratios ( $^{13}\text{C}/^{12}\text{C}$ ) of the relevant metabolites should whenever possible be measured each time an elevated parameter of the steroid profile is estimated from the Screening Procedure or Confirmation Procedure and reported to the Testing Authority as having been determined. If the Laboratory does not have the capability to conduct such testing, the *Samples* are to be securely transferred ensuring the Chain of Custody to another Laboratory with the requisite capability.
- The results of the IRMS analysis and/or of the steroid profile measured by GC/MS shall be used to draw conclusions as to whether a doping violation may have been committed. If the IRMS study does not readily indicate exogenous administration, the result should be reported as “inconclusive” and if necessary further longitudinal studies performed.
- When available, the athlete’s previous tests on record at the Testing Authority should be accessed and the corresponding steroid profile data requested from the relevant Laboratory. These results should be examined and considered together with the existing evidence (longitudinal study).

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- If, for any reason, an IRMS analysis cannot be carried out satisfactorily (e.g. insufficient volume of urine, amount of analyte too low to enable a valid measurement) or the examination of previous test results raises suspicions due to unstable profile values, up to three further unannounced tests should be carried out, preferably within a three months period following the report of the suspicious analytical result. There should be a minimum total of three results, other than the abnormal *Sample*, of either past or post data. A *Sample* in which the elevated parameter is again measured is to be analysed by IRMS as described above. In difficult cases longer monitoring may be required.

### 5. Evaluation of longitudinal studies:

In males, the individual T/E values have been shown to vary from their mean value by less than 30% (screening values). In females, a low concentration of some urinary steroids such as epitestosterone and testosterone, close to the limit of detection using current analytical methods occurs. Normal variation of up to 60% may be expected. The individual basal T/E value should be determined from at least three test results, excluding the suspicious result under consideration. The mean, standard deviation and coefficient of variation (expressed in percent) should be calculated for those three basal values. If the suspicious test result, when compared to the basal value using appropriate statistical evaluation is found to be significantly different, that will constitute a proof of the administration of a source of testosterone. It is understood that the basal value may be calculated from previous screening test results. The comparison of screening results and confirmed results is acceptable.

The same reasoning applies to any other parameter of the steroid profile which has been estimated to be in an amount exceeding the ranges of values normally found in humans.

### 6. Other parameters:

Other parameters such as the ratio of urinary testosterone to Lutenising Hormone (T/LH) and the androsterone to testosterone ratio (A/T) may be used to provide extra information to help determine the use of some substances especially injected testosterone and many of its esters. A high T/LH ratio may be used as ancillary evidence. The A/T ratio which has markedly changed from the "normal" value found for an individual during a longitudinal study may indicate which type of substance has been used. A change to high value can indicate testosterone use and a change to low values may indicate the use of testosterone precursors such as DHEA. However, any administration of testosterone and of its precursors, androstenedione or DHEA will not necessarily alter the excretion of LH and epitestosterone glucuronide.



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## 7. Examples of specific urinary metabolites potentially altered by the administration of “endogenous steroids”;

Urinary steroid	Steroid administered
Testosterone (G)	Testosterone, androstenedione, DHEA
Epitestosterone (G)	Epitestosterone
T/E (G)	Testosterone, androstenedione, DHEA
Androsterone (G)	Testosterone, DHT, androstenedione, DHEA and androstenediol
Etiocholanolone (G)	Testosterone, androstenedione, DHEA and androstenediol
DHEA (G) (S)	DHEA
6 $\alpha$ -OH Androstenedione (G)	Androstenedione
6 $\beta$ -OH Androsterone (G)	Androstenedione
6 $\beta$ -OH Etiocholanolone (G)	Androstenedione
6 $\beta$ -OH Epiandrosterone (S)	Androstenedione
7 $\beta$ -OH DHEA/16 $\alpha$ -OH Androsterone (S)	DHEA
7-OH DHEA, 7 keto DHEA	7 keto DHEA

\* G indicates the glucuronide and S indicates sulphate conjugation.

The official text of the technical document Reporting and Evaluation Guidance for Testosterone, Epitestosterone, T/E Ratio and other Endogenous Steroids shall be maintained by WADA and shall be published in English and French. In the event of any conflict between the English and French versions, the English version shall prevail.

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# The World Anti-Doping Code

# **INTERNATIONAL STANDARD FOR LABORATORIES**

**Version 3.0**

June 2003



## PREAMBLE

The World Anti-Doping Code *International Standard* for Laboratories is a mandatory level 2 *International Standard* developed as part of the World Anti-Doping Program.

The basis for the *International Standard* for Laboratories is the relevant Sections in the Olympic Movement Anti-Doping Code. An expert group, together with a WADA Laboratory Accreditation Committee, has prepared the document and drafts have been circulated for initial review and comment from all IOC accredited doping Laboratories and the IOC Sub-Commission on Doping and Biochemistry of Sport.

Version 1.0 of the *International Standard* for Laboratories was circulated to *Signatories*, governments and accredited laboratories for review and comments in November 2002. Version 2.0 was based on the comments and proposals received from these stakeholders.

All *Signatories*, governments and Laboratories were consulted and have had the opportunity to review and provide comments to version 2.0. This draft version 3.0 was presented for approval to the WADA Executive Committee on June 7<sup>th</sup> 2003.

The *International Standard* for Laboratories will come into effect on January 1<sup>st</sup> 2004.

Currently, Laboratories are accredited by the International Olympic Committee (IOC). As part of the transition of the program from existing IOC accreditation to WADA accreditation, accreditation bodies shall require the Laboratories to which they grant and maintain accreditation to comply with the requirements of the *International Standard* for Laboratories and ISO/IEC 17025 by January 1<sup>st</sup>, 2004. For Laboratories moving from IOC to WADA accreditation (see Section 4.1.7), an internal audit before January 1<sup>st</sup>, 2004 shall be deemed compliant with the *International Standard* for Laboratories. The next ISO surveillance or re-accreditation audit conducted by the national accrediting body in 2004 shall document compliance with the *International Standard* for Laboratories. Laboratories seeking initial WADA accreditation shall have an on-site accreditation audit by their national accrediting body compliant with this standard before receiving WADA accreditation.

The official text of the Code shall be maintained by WADA and shall be published in English and French. In the event of any conflict between the English and French versions, the English version shall prevail.

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# PART ONE: INTRODUCTION, CODE PROVISIONS AND DEFINITIONS

## 1.0 Introduction, Scope and References

The main purpose of the *International Standard for Laboratories* is to ensure laboratory production of valid test results and evidentiary data and to achieve uniform and harmonized results and reporting from all accredited *Doping Control Laboratories*.

The *International Standard for Laboratories* includes requirements for WADA accreditation of doping laboratories, operating standards for laboratory performance and description of the accreditation process.

The *International Standard for Laboratories*, including all Annexes and Technical Documents, is mandatory for all *Signatories* to the *Code*.

The World Anti-Doping Program encompasses all of the elements needed in order to ensure optimal harmonization and best practice in international and national anti-doping programs. The main elements are: the *Code* (Level 1), *International Standards* (Level 2), and Models of Best Practice (Level 3).

In the introduction to the World Anti-Doping *Code* (*Code*), the purpose and implementation of the *International Standards* are summarized as follows:

"*International Standards* for different technical and operational areas within the anti-doping program will be developed in consultation with the *Signatories* and governments and approved by WADA. The purpose of the *International Standards* is harmonization among *Anti-Doping Organizations* responsible for specific technical and operational parts of the anti-doping programs. Adherence to the *International Standards* is mandatory for compliance with the *Code*. The *International Standards* may be revised from time to time by the WADA Executive Committee after reasonable consultation with the *Signatories* and governments. Unless provided otherwise in the *Code*, *International Standards* and all revisions shall become effective on the date specified in the *International Standard* or revision."

Compliance with an *International Standard* (as opposed to another alternative standard, practice or procedure) shall be sufficient to conclude that the procedures covered by the *International Standard* were performed properly.

This document sets out the requirements for *Doping Control Laboratories* that wish to demonstrate that they are technically competent, operate an effective quality management system, and are able to produce forensically valid results. *Doping Control Testing* involves the detection, identification, and in some cases demonstration of the presence greater than a threshold concentration of drugs and other substances deemed to be prohibited by the list of *Prohibited Substances* and *Prohibited Methods* (*The Prohibited List*) in human biological fluids or tissues.

The Laboratory accreditation framework consists of two main elements: Part Two of the standard: the Laboratory accreditation requirements and operating standards; and Part Three: the Annexes and Technical Documents. Part Two describes the requirements necessary to obtain WADA recognition and the procedures involved to fulfill the requirements. It also contains an application of the ISO/IEC 17025 standard to the field of *Doping Control*. The purpose of this section of the document is to facilitate consistent application and assessment of the ISO/IEC 17025 and the specific WADA requirements for *Doping Control* by accreditation bodies that operate in accordance with ISO/IEC Guide 58. The *International Standard* also sets forth the requirements for *Doping Control Laboratories* when adjudication results as a consequence of an *Adverse Analytical Finding*.

Part Three of the Standard includes all Annexes. Annex A describes the WADA Proficiency Testing Program, including performance criteria necessary to maintain good standing in proficiency testing. Annex B describes the ethical standards required for continued WADA recognition of the Laboratory. Annex C is a list of Technical Documents. Technical Documents are issued, modified, and deleted by WADA from time to time and provide direction to the Laboratories on specific technical issues. Once promulgated, Technical Documents become part of the *International Standard* for Laboratories. The incorporation of the provisions of the Technical Documents into the Laboratory's quality management system is mandatory for WADA accreditation.

In order to harmonize the accreditation of Laboratories to the requirements of ISO/IEC 17025 and the WADA-specific requirements for recognition, it is expected that national accreditation bodies will use this standard, including the annexes, as a reference document in their accreditation audit process.

Terms defined in the *Code*, which are included in this standard, are written in italics. Terms, which are defined in this standard, are underlined.

## References

These following references were consulted in the development of this document. The specific requirements and concepts of these documents do not supersede or otherwise change the requirements stated in the *International Standard* for Laboratories.

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International Laboratory Accreditation Cooperation (ILAC) Document G-7:1996. Accreditation Requirements and Operating Criteria for Horseracing Laboratories.

ILAC Document G-15:2001. Guidance for Accreditation to ISO/IEC 17025

ILAC Document G-17:2002. Introducing the Concept of Uncertainty of Measurement in Testing in Association with the Application of the Standard ISO/IEC 17025.

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ILAC Document P-10:2002. ILAC Policy on Traceability of Measurement Results.

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Society of Forensic Toxicology and American Academy of Forensic Sciences, Toxicology Section, 2002 (Draft). Forensic Toxicology Laboratory Guidelines.

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World Anti-Doping Code

## 2.0 Code Provisions

The following articles in the *Code* directly address the *International Standard for Laboratories*:

### Code Article 3.2 Methods of Establishing Facts and Presumptions

**3.2.1** WADA-accredited Laboratories are presumed to have conducted *Sample* analysis and custodial procedures in accordance with the *International Standard* for laboratory analysis. The *Athlete* may rebut this presumption by establishing that a departure from the *International Standard* occurred. If the *Athlete* rebuts the preceding presumption by showing that a departure from the *International Standard* occurred, then the *Anti-Doping Organization* shall have the burden to establish that such departure did not cause the *Adverse Analytical Finding*.

### Code Article 6 Analysis of Samples

*Doping Control Samples* shall be analyzed in accordance with the following principles:

**6.1 Use of Approved Laboratories** *Doping Control Samples* shall be analyzed only in WADA-accredited laboratories or as otherwise approved by WADA. The choice of the WADA-accredited laboratory (or other method approved by WADA) used for the *Sample* analysis shall be determined exclusively by the *Anti-Doping Organization* responsible for results management.

[Comment: The phrase "or other method approved by WADA" is intended to cover, for example, mobile blood Testing procedures which WADA has reviewed and considers to be reliable.]

**6.2 Substances Subject to Detection.** *Doping Control Samples* shall be analyzed to detect *Prohibited Substances* and *Prohibited Methods* identified on the *Prohibited List* and other substances as may be directed by WADA pursuant to Article 4.5 (Monitoring Program).

**6.3 Research on Samples.** No *Sample* may be used for any purpose other than the detection of substances (or classes of substances) or methods on the *Prohibited List*, or as otherwise identified by WADA pursuant to Article 4.5 (Monitoring Program), without the *Athlete's* written consent.

**6.4 Standards for Sample Analysis and Reporting.** Laboratories shall analyze *Doping Control Samples* and report results in conformity with the *International Standard* for Laboratories analysis.

**Code Article 13.5 Appeals from Decisions Suspending or Revoking Laboratory Accreditation** Decisions by WADA to suspend or revoke a Laboratory's WADA accreditation may be appealed only by that Laboratory with the appeal being exclusively to CAS.

**Code Article 14.1 Information Concerning Adverse Analytical Findings and Other Potential Anti-Doping Rule Violations.** An *Athlete* whose *Sample* has resulted in an *Adverse Analytical Finding*, or an *Athlete* or other *Person* who may have violated an anti-doping rule, shall be notified by the *Anti-Doping Organization* with results management responsibility as provided in Article 7 (Results Management). The *Athlete's National Anti-Doping Organization* and International Federation and WADA shall also be notified not later than the completion of the process described in Articles 7.1 and 7.2. Notification shall include: the *Athlete's* name, country, sport and discipline within the sport; whether the test was *In-Competition* or *Out-of-Competition*, the date of *Sample* collection and the analytical result reported by the laboratory. The same *Persons* and *Anti-Doping Organizations* shall be regularly updated on the status and findings of any review or proceedings conducted pursuant to Articles 7 (Results Management), 8 (Right to a Fair Hearing) or 13 (Appeals), and, in any case in which the period of *Ineligibility* is eliminated under Article 10.5.1 (*No Fault or Negligence*), or reduced under Article 10.5.2 (*No Significant Fault or Negligence*), shall be provided with a written reasoned decision explaining the basis for the elimination or reduction. The recipient organizations shall not disclose this information beyond those *Persons* within the organization with a need to know until the *Anti-Doping Organization* with

results management responsibility has made public disclosure or has failed to make public disclosure as required in Article 14.2.

## 3.0 Terms and definitions

### 3.1 Code defined Terms

**Adverse Analytical Finding:** A report from a Laboratory or other approved *Testing* entity that identifies in a *Specimen* the presence of a *Prohibited Substance* or its *Metabolites* or *Markers* (including elevated quantities of endogenous substances) or evidence of the *Use* of a *Prohibited Method*.

**Anti-Doping Organization:** A *Signatory* that is responsible for adopting rules for, initiating, implementing or enforcing any part of the *Doping Control* process. This includes, for example, the International Olympic Committee, the International Paralympic Committee, *Major Event Organizations* that conduct *Testing* at their *Events*, WADA, International Federations, and *National Anti-Doping Organizations*.

**Athlete:** For purposes of *Doping Control*, any *Person* who participates in sport at the international level (as defined by each International Federation) or national level (as defined by each *National Anti-Doping Organization*) and any additional *Person* who participates in sport at a lower level if designated by the *Person's National Anti-Doping Organization*. For purposes of anti-doping information and education, any *Person* who participates in sport under the authority of any *Signatory*, government, or other sports organization accepting the *Code*.

**Code:** The World Anti-Doping Code.

**Doping Control:** The process including test distribution planning, *Sample* collection and handling, Laboratory analysis, results management, hearings and appeals.

**Event:** A series of individual *Competitions* conducted together under one ruling body (e.g., the Olympic Games, FINA World Championships, or Pan American Games).

**In-competition:** For purposes of differentiating between *In-competition* and *Out-of-Competition Testing*, unless provided otherwise in the rules of an International Federation or other relevant *Anti-Doping Organization*, an *In-Competition* test is a test where an *Athlete* is drawn for *Testing* in connection with a specific *Competition*.

**International Standard:** A standard adopted by WADA in support of the *Code*. Compliance with an *International Standard* (as opposed to another alternative standard, practice or procedure) shall be sufficient to conclude that the procedures covered by the *International Standard* were performed properly.

**Marker:** A compound, group of compounds or biological parameters that indicates the *Use* of a *Prohibited Substance* or *Prohibited Method*.

**Metabolite:** Any substance produced by a biotransformation process.

**National Anti-Doping Organization:** The entity(ies) designated by each country as possessing the primary authority and responsibility to adopt and implement anti-doping rules, direct the collection of *Samples*, the management of test results, and the conduct of hearings, all at the national level. If this designation has not been made by the competent public authority(ies), the entity shall be the country's *National Olympic Committee* or its designee.

**National Olympic Committee:** The organization recognized by the International Olympic Committee. The term *National Olympic Committee* shall also include the National Sport Confederation in those countries where the National Sport Confederation assumes typical *National Olympic Committee* responsibilities in the anti-doping area.

**Out-of-Competition:** Any *Doping Control* which is not *In-competition*.

**Person:** A natural person or an organization or other entity.

**Prohibited List:** The List identifying the *Prohibited Substances* and *Prohibited Methods*.

**Prohibited Method:** Any method so described on the *Prohibited List*.

**Prohibited Substance:** Any substance so described on the *Prohibited List*.

**Publicly Disclose or Publicly Report:** To disseminate or distribute information to the general public or *Persons* beyond those *Persons* entitled to earlier notification in accordance with Article 14.

**Sample/Specimen:** Any biological material collected for the purposes of *Doping Control*.

**Signatories:** Those entities signing the *Code* and agreeing to comply with the *Code*, including the International Olympic Committee, International Federations, International Paralympic Committee, *National Olympic Committees*, National Paralympic Committees, *Major Event Organizations*, *National Anti-Doping Organizations*, and WADA.

**Testing:** The parts of the *Doping Control* process involving test distribution planning, *Sample* collection, *Sample* handling, and *Sample* transport to the Laboratory.

**Use:** The application, ingestion, injection or consumption by any means whatsoever of any *Prohibited Substance* or *Prohibited Method*.

**WADA:** The World Anti-Doping Agency.



### 3.2 Defined Terms from the *International Standard for Laboratories*

**Aliquot:** A portion of the *Sample* of biological fluid or tissue (e.g., urine, blood, etc.) obtained from the *Athlete* used in the testing process.

**Certified Reference Material:** Reference Material, accompanied by a certificate, one or more whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

**Confirmation Procedure:** An analytical test procedure whose purpose is to identify the presence of a specific *Prohibited Substance* in a *Sample*. [Comment: A *Confirmation Procedure* may also indicate a quantity of *Prohibited Substance* greater than a threshold value or quantify the amount of a *Prohibited Substance* in a *Sample*.]

**Flexible Accreditation:** Approval for a Laboratory to make restricted modifications in the scope of the accreditation without the involvement of the national accreditation body before the modifications are implemented

**Intermediate Precision,  $s_z$ :** Variation in results observed when one or more factors, such as time, equipment, and operator are varied within a Laboratory with  $i$  denoting the number of factors varied.

**Laboratory Internal Chain of Custody:** Documentation of the sequence of *Persons* in possession of the *Sample* and any portions of the *Sample* taken for *Testing*. [Comment: *Laboratory Internal Chain of Custody* is generally documented by a written record of the date, location, action taken, and the individual performing an action with a *Sample* or *Aliquot*.]

**Laboratory:** An accredited laboratory applying test methods and processes to provide evidentiary data for the detection and, if applicable, quantification of a Threshold Substance on the *Prohibited List* in urine and other biological *Samples*.

**Laboratory Documentation Packages:** The material produced by the Laboratory to support the finding of an *Adverse Analytical Finding* as set forth in the WADA Technical Document for Laboratory Documentation Packages.

**Minimum Required Performance Limit:** A concentration of a *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method* that a doping Laboratory is expected to reliably detect in the routine daily operation of the Laboratory. See Technical Document Minimum Required Performance Limits for Detection of Prohibited Substances.

**Non-threshold Substance:** A substance listed on the *Prohibited List* for which the documentable detection of any amount is considered an anti-doping rule violation.

**Presumptive Analytical Finding:** The status of a *Sample* test result for which there is an adverse screening test, but a confirmation test has not been performed.

**Reference Collection:** A collection of samples of known origin that may be used in the determination of the identity of an unknown substance. For example, a well characterized sample obtained from a verified administration study in which scientific documentation of the identity of *Metabolite(s)* can be demonstrated.

**Reference Material:** Material or substance one or more of whose properties are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method or for assigning values to materials.

**Repeatability,  $s_r$ :** Variability observed within a laboratory, over a short time, using a single operator, item of equipment, etc.

**Reproducibility,  $s_R$ :** Variability obtained when different laboratories analyze the same *Sample*.

**Revocation:** The permanent withdrawal of a *Laboratory's* WADA accreditation.

**Screening Procedure:** An analytical test procedure whose purpose is to identify those *Samples* which are suspicious with respect to containing a *Prohibited Substance* or *Metabolite* or *Marker* of a *Prohibited Method* and which require additional confirmation testing.

**Split Sample:** Division of a *Sample* taken for testing into two portions at collection, usually designated "A" and "B."

**Suspension:** The temporary withdrawal of a *Laboratory's* WADA accreditation.

**Testing Authority:** The International Olympic Committee, World Anti-Doping Agency, International Federation, National Sport Organization, *National Anti-Doping Organization*, *National Olympic Committee*, *Major Event Organization*, or other authority defined by the Code responsible for *Sample* collection and transport either *In-Competition* or *Out-of-Competition* and/or for management of the test result.

**Threshold Substance:** A substance listed in the *Prohibited List* for which the detection of an amount in excess of a stated threshold is considered an *Adverse Analytical Finding*.

## **PART TWO: LABORATORY ACCREDITATION REQUIREMENTS AND OPERATING STANDARDS**

### **4.0 Requirements for WADA accreditation**

#### **4.1 Initial WADA accreditation**

This section describes the specific requirements for the initial WADA accreditation of the laboratory. All the requirements must be fulfilled in order to obtain an initial WADA accreditation. For some of the requirements, the laboratory has to demonstrate compliance during the probationary period and for other requirements compliance will be checked and controlled based on an accreditation audit (ref. 5.1, 5.2 and 5.3).

##### **4.1.1 ISO/IEC 17025**

The laboratory shall be accredited by a relevant national accreditation body according to ISO/IEC 17025 with primary reference to the interpretations and applications of the ISO/IEC 17025 requirements as they are described in Application of ISO/IEC 17025 to the Analysis of *Doping Control Samples* (Section 5). The ISO/IEC 17025 accreditation must be obtained before the initial WADA accreditation will be given.

##### **4.1.2 Letter of support**

The laboratory shall provide an official letter of support from the relevant national public authority responsible for the national anti-doping program, if any, or a similar letter of support from the *National Olympic Committee* or *National Anti-Doping Organization*. The letter of support shall contain as a minimum:

- Guarantee of sufficient financial support annually for a minimum of 3 years
- Guarantee of sufficient numbers of *Samples* annually for 3 years
- Guarantee of provision of necessary analytical facilities and instrumentation, where applicable

In addition, any explanation of exceptional circumstances shall be given due consideration by WADA. The three year letter of support does not in any way require exclusive support for only one laboratory.

Letters of support from international sport organizations such as International Federations could also be provided in addition to the above mentioned letters.

If the laboratory as an organization is linked to host organizations, (e.g. universities, hospitals...) an official letter of support from the host organizations shall be provided which should include the following information:

- Documentation of the administrative support for the laboratory
- Financial support for the laboratory, if relevant

- Support for the research and development activities
- Guarantee of provision of necessary analytical facilities and instrumentation

#### **4.1.3 Code of Ethics**

The laboratory shall sign and comply with the provision in the Code of Ethics (Annex B) which are relevant for a laboratory in the probationary period.

#### **4.1.4 Proficiency testing program**

During the probationary period the laboratory shall successfully analyze at a minimum four sets of proficiency testing samples containing at a minimum five samples per set.

The final accreditation test shall assess both the scientific competence and the capability of the laboratory to manage multiple *Samples*.

#### **4.1.5 Sharing of knowledge**

The laboratory shall demonstrate during the probationary period its willingness and ability to share knowledge with other *WADA Accredited Laboratories*. A description of this sharing is provided in the Code of Ethics (Annex B).

#### **4.1.6 Research**

The laboratory shall demonstrate in its budget an allocation to research and development activities in the field of *Doping Control* of at least 7% of the annual budget for the initial 3-year period. The research activities can either be conducted by the laboratory or in cooperation with other *WADA-accredited Laboratories* or other research organizations.

#### **4.1.7 Initial accreditation of Laboratories holding IOC accreditation**

*Laboratories* accredited by the IOC in 2003 and which successfully complete the joint 2003 IOC/WADA re-accreditation test and at a minimum conduct an internal audit against Section 5 of the *Internal Standard for Laboratories* will receive WADA accreditation in 2004. The *International Standards for Laboratories* requirements will be fully in effect on January 1<sup>st</sup>, 2004. *Laboratories* that are downgraded or fail the 2003 IOC/WADA re-accreditation test will have their accreditation suspended or revoked by WADA in accordance with Section 6.4.8. Laboratories which have applied for, but have not received, IOC accreditation will complete their probationary period under the *International Standards for Laboratories*.

### **4.2 Maintaining WADA Accreditation**

This section describes the specific requirements for a *WADA* re-accreditation of the *Laboratory*.

#### **4.2.1 ISO/IEC 17025 accreditation**

The *Laboratory* shall document a valid accreditation from the national accreditation body according to ISO/IEC 17025 with primary reference to the interpretations and applications of the ISO/IEC 17025 requirements as described in the Application of ISO/IEC 17025 to Analysis of *Doping Control Samples* (Section 5).

#### **4.2.2 Flexible Accreditation**

WADA accredited Laboratories may add or modify scientific methods or add analytes without the need for approval by the body that completed the ISO/IEC 17025 accreditation of that Laboratory. Any analytical method or procedure must be properly selected and validated and included in the scope of the Laboratory at the next ISO audit if the method is used for analysis of *Doping Control Samples*.

#### **4.2.3 Letter of support**

The Laboratory shall provide a renewed official letter of support from the relevant national public authority responsible for the national anti-doping program, if any, or a similar letter of support from the *National Olympic Committee* or *National Anti-Doping Organization* in years in which the Laboratory undergoes an ISO re-accreditation audit. The renewed letter of support shall contain as a minimum:

- Guarantee of sufficient financial support annually for a minimum of 3 years
- Guarantee of sufficient numbers of *Samples* annually
- Guarantee of provision of necessary analytical facilities and instrumentation, where applicable

Any explanation of exceptional circumstances shall be given due consideration by WADA. The letter of support does not in any way require exclusive support for only one Laboratory.

Letters of support from international sport organizations such as International Federations could also be provided in addition to the above mentioned letters.

If the Laboratory as an organization is linked to host organizations (e.g. university, hospital...), an official letter of support from the host organizations shall be renewed for each year in which the Laboratory undergoes a ISO re-accreditation audit and shall include the following information:

- Documentation of the administrative support for the Laboratory
- Financial support for the Laboratory, if relevant
- Guarantee of provision of necessary analytical facilities and instrumentation
- Support for the research activities

#### **4.2.4 Minimum number of testing Samples**

The Laboratory shall periodically provide, at the request of WADA a report documenting all test results reported in a format to be specified by WADA.

In order to maintain proficiency, WADA-accredited Laboratories are required to analyze a minimum of 1500 *Doping Control Samples* per year that are provided by a Testing Authority. If the Laboratory fails to analyze this number of *Samples*, accreditation will be suspended or revoked, dependent on the circumstances.

#### **4.2.5 Proficiency testing program**

The Laboratories are required to successfully participate in the WADA Proficiency Testing program. The program is described in more detail in Annex A.

#### **4.2.6 Reporting**

The Laboratory shall simultaneously report to WADA and the relevant International Federation all *Adverse Analytical Findings* that have been reported to a Testing Authority. All reporting shall be in accord with the confidentiality requirements of the *Code*.

#### **4.2.7 Code of Ethics**

The Laboratory shall provide documentation of compliance with the provisions of the Code of Ethics (Annex B) relevant for a WADA accredited Laboratory. The Laboratory Director shall send a letter of compliance to WADA every year.

#### **4.2.8 Sharing of knowledge**

The Laboratory shall demonstrate their willingness and ability to share knowledge with other WADA Accredited Laboratories. A description of this sharing is provided in the Code of Ethics (Annex B).

#### **4.2.9 Research**

The Laboratory shall maintain an updated 3-year plan for research and development in the field of *Doping Control*, including an annual budget in this area.

The Laboratory should document the publication of results of the research in relevant scientific papers in the peer-reviewed literature. These documents shall be made available to WADA upon request. The Laboratory may also demonstrate a research program by documenting successful or pending applications for research grants.

### **4.3 Special Requirements for Major Events**

The Laboratory support for the Olympic Games and other major *Events* may be such that the accredited Laboratory facilities are not adequate. This may require relocation of the Laboratory to a new facility, the addition of personnel, or the acquisition of additional equipment. The Laboratory Director of the WADA-accredited Laboratory designated to perform the testing shall be responsible to ensure that the quality management system is maintained.

#### **4.3.1 Satellite facility of an accredited Laboratory**

If the Laboratory is required to move or extend its operation temporarily to a new physical location, the Laboratory must demonstrate a valid ISO/IEC 17025 accreditation with primary compliance with the Application of ISO/IEC 17025 to the Analysis of *Doping Control Samples* for the new facility ("satellite facility").

Any methods or equipment unique to the satellite facility must be validated prior to the satellite facility accreditation audit. Any changes to methods or other procedures in the quality manual must also be validated prior to the audit.

#### **4.3.2 Personnel**

The Laboratory shall report to WADA any senior personnel (e.g., certifying scientists, quality system management staff, supervisors, etc.) temporarily working in the Laboratory. The Laboratory Director shall ensure that these personnel are adequately trained in the methods, policies, and procedures of the Laboratory. Particular emphasis should be given to the Code of Ethics and the confidentiality of the results management process. Adequate documentation of training of these temporary employees should be maintained by the Laboratory.

#### **4.3.3 Proficiency testing**

WADA may, at its sole discretion, submit proficiency testing samples to the Laboratory for analysis. The samples shall be analyzed by the same methods used in the testing of *Samples* from a Testing Authority. These samples may be part of the ISO/IEC 17025 audit in conjunction with the national accrediting body. Failure(s) to successfully complete the proficiency test will be considered by WADA in deciding whether to accredit the Laboratory. In the event of an unacceptable report, the Laboratory shall document the changes instituted to remedy the failure.

The proficiency testing process should include any additional personnel that are added to the staff for the major *Event*. The samples should be analyzed using the protocols and procedures that will be used for analysis of *Samples* for the *Event*.

#### **4.3.4 Reporting**

The Laboratory shall document that the reporting of test results maintains confidentiality.

## **5.0 Application of ISO 17025 to the Analysis of Doping Control Samples**

### **5.1 Introduction and Scope**

This section of the document is intended as an application as described in Annex B.4 (Guidelines for establishing applications for specific fields) of ISO/IEC 17025 for the field of *Doping Control*. Any aspect of testing or management not specifically discussed in this document shall be governed by ISO/IEC 17025 and, where applicable, by ISO 9001. The application focuses on the specific parts of the processes that are critical with regard to the quality of the laboratory's performance as a *Doping Control Laboratory*. These processes have been determined to be critical to the defined ISO 17025 criteria and are therefore determined to be significant in the evaluation and accreditation process.

This section introduces the specific performance standards for a *Doping Control Laboratory*. The conduct of testing is considered a process within the definitions of ISO 9001. Performance standards are defined according to a process model where the *Doping Control Laboratory* practice is structured into three main categories of processes:

- Analytical and technical processes
- Management processes
- Support processes

Wherever possible, the application will follow the format of the ISO 17025 document. The concepts of the quality management system, continuous improvement, and customer satisfaction included in ISO 9001 have been included.

## 5.2 Analytical and Technical Processes

### 5.2.1 Receipt of Samples

- 5.2.1.1 Samples may be received by any method authorized by the *International Standard for Testing*.
- 5.2.1.2 The transport container shall first be inspected and any irregularities recorded.
- 5.2.1.3 The name and signature (or other means of identification and recording) of the *Person* delivering or transferring custody of the shipped *Samples*, the date, the time of receipt, and the name and signature of the Laboratory representative receiving the *Samples*, shall be documented as part of the Laboratory Internal Chain of Custody record.

### 5.2.2 Handling of Samples

- 5.2.2.1 The Laboratory shall have a system to uniquely identify the *Samples* and associate each *Sample* with the collection document or other external chain of custody.

5.2.2.2 The Laboratory shall have Laboratory Internal Chain of Custody procedures to maintain control of and accountability for *Samples* from receipt through final disposition of the *Samples*. The procedures must incorporate the concepts presented in the WADA Technical Document for Laboratory Internal Chain of Custody (Annex C).

5.2.2.3 The Laboratory shall observe and document conditions that exist at the time of receipt that may impact on the integrity of a *Sample* report. For example, irregularities noted by the Laboratory should include, but are not limited to:

- *Sample* tampering is evident.
- *Sample* is not sealed with tamper-resistant device or seal upon receipt.
- *Sample* is without a collection form (including *Sample* identification code) or a blank form is received with the *Sample*.
- *Sample* identification is unacceptable. For example, the number on the bottle does not match the *Sample* identification number on the form.
- *Sample* volume is extremely low



5.2.2.4 The Laboratory should notify and seek advice from the Testing Authority regarding rejection and testing of *Samples* for which irregularities are noted.

5.2.2.5 The Laboratory shall retain the A and B *Sample(s)* for a minimum of two (2) weeks after the Testing Authority receives a negative report. The *Samples* shall be retained under appropriate conditions.

*Samples* with irregularities shall be held for a minimum of two (2) weeks following the report to the Testing Authority.

5.2.2.6 The Laboratory shall retain the *Sample(s)* with an *Adverse Analytical Finding* for a minimum of three months after the Testing Authority receives the final analytical (A or B *Sample*) report. The *Sample* shall be stored under appropriate conditions during the long term storage.

5.2.2.7 If the Laboratory has been informed by the Testing Authority that the analysis of a *Sample* is challenged or disputed, the *Sample* shall be retained under appropriate conditions and all the records pertaining to the *Testing* of that *Sample* shall be stored until completion of any challenges.

5.2.2.8 The Laboratory shall maintain a policy pertaining to retention, release, and disposal of *Samples* or Aliquots.

5.2.2.9 The Laboratory shall maintain custody information on the transfer of *Samples*, or portions thereof to another Laboratory.

### **5.2.3 Sampling and Preparation of Aliquots for Testing**

5.2.3.1 The Laboratory shall maintain Laboratory Internal Chain of Custody procedures for control of and accountability for all Aliquots from preparation through disposal. The procedures must incorporate the concepts presented in the WADA Technical Document for Laboratory Internal Chain of Custody.

5.2.3.2 Before the initial opening of a *Sample* bottle, the device used to ensure integrity of the *Sample* (e.g., security tape or a bottle sealing system) shall be inspected and the integrity documented.

5.2.3.3 The Aliquot preparation procedure for any Screening Procedure or Confirmation Procedure shall ensure that no risk of contamination of the *Sample* or Aliquot exists.

### **5.2.4 Testing**

#### **5.2.4.1 Urine integrity testing**

5.2.4.1.1 The Laboratory must have a written policy establishing the procedures and criteria for *Sample* integrity tests.

5.2.4.1.2 The Laboratory should note any unusual condition of the urine – for example: color, odor, or foam. Any unusual conditions should be recorded and included as part of the report to the Testing Authority.

5.2.4.1.3 The Laboratory shall test for the pH and specific gravity as urine integrity parameters on the "A" *Sample*. Other tests may be performed if requested by the Testing Authority and approved by WADA

#### 5.2.4.2 Urine screen testing

5.2.4.2.1 The Screening Procedure(s) shall detect the *Prohibited Substance(s)* or *Metabolite(s)* of *Prohibited Substance(s)*, or *Marker(s)* of the *Use of a Prohibited Substance or Method* for all substances listed in the *Out-of-Competition* or *In-competition* Section of the *Prohibited List* as appropriate for which there is a WADA-accepted screening method. WADA may make specific exceptions to this section.

5.2.4.2.2 The Screening Procedure shall be performed with a WADA-accepted validated method that is appropriate for the substance or method being tested. The criteria for accepting a screening result and allowing the testing of the *Sample* to proceed must be scientifically valid.

5.2.4.2.3 All screening assays shall include negative and positive controls in addition to the *Samples* being tested.

5.2.4.2.4 For analytes that must exceed a threshold for reporting as an *Adverse Analytical Finding*, appropriate controls shall be included in the screening assay. Screening Procedures for Threshold Substances are not required to meet quantitative or uncertainty requirements.

#### 5.2.4.3 Urine confirmation testing

All Confirmation Procedures must be documented and meet applicable uncertainty requirements. The objective of a Confirmation Procedure is to ensure the identification and/or quantification and to exclude any technical deficiency in the Screening Procedure. Since the objective of the confirmation assay is to accumulate additional information regarding an adverse finding, a Confirmation Procedure should have greater selectivity/discrimination than a Screening Procedure.

#### 5.2.4.3.1 "A" Sample Confirmation

5.2.4.3.1.1 Presumptive identification from a Screening Procedure of a *Prohibited Substance*, *Metabolite(s)* of a *Prohibited Substance*, or *Marker(s)* of the *Use of a Prohibited Substance* or *Method* must be confirmed using a second Aliquot(s) taken from the original "A" *Sample*.

5.2.4.3.1.2 Mass spectrometry coupled to either gas or liquid chromatography is the method of choice for confirmation of *Prohibited Substances*, *Metabolite(s)* of a *Prohibited Substance*, or *Marker(s)* of the *Use of a Prohibited Substance* or *Method*. GC/MS or HPLC/MS are acceptable for both Screening Procedures and Confirmation Procedures for a specific analyte.

5.2.4.3.1.3 Immunoassay for confirmation of prohibited proteins, peptides, mimetics, and analogues or *Marker(s)* of their *Use* is permitted. The immunoassay used for confirmation must use a procedure with a different antibody that should recognise a different epitope of the peptide/protein than the assay used for screening.

5.2.4.3.1.4 The Laboratory must have a policy to define those circumstances where the confirmation testing of an "A" *Sample* may be repeated (e.g., batch quality control failure). Each repeat confirmation must be documented and be completed on a new Aliquot of the "A" *Sample*.

5.2.4.3.1.5 The Laboratory is not required to confirm every *Prohibited Substance* that is identified by the Screening Procedures. The decision on the prioritization on order of confirmation(s) should be made in cooperation with the Testing Authority and the decision documented. In addition, no Certificate of Analysis or final written Test Report incorporating a Presumptive Analytical Finding shall be issued.

#### 5.2.4.3.2 "B" Sample Confirmation

5.2.4.3.2.1 In those cases where confirmation of a *Prohibited Substance*, *Metabolite(s)* of a *Prohibited Substance*, or *Marker(s)* of the *Use of a Prohibited Substance* or *Method* is requested in the "B" *Sample*, the "B" *Sample* analysis should occur as soon as possible and should be completed within thirty (30) days of notification of an "A" *Sample Adverse Analytical Finding*.

5.2.4.3.2.2 The "B" *Sample* confirmation must be performed in the same Laboratory as the "A" *Sample* confirmation. A different

analyst must perform the "B" analytical procedure. The same individual(s) that performed the "A" analysis may perform instrumental set up and performance checks and verify results.

5.2.4.3.2.3 The *B Sample* result must confirm the *A Sample* identification for the *Adverse Analytical Finding* to be valid. The mean value for the *B Sample* finding for Threshold Substances is required to exceed that threshold including consideration of uncertainty.

5.2.4.3.2.4 The *Athlete and/or a representative*, a representative of the entity responsible for *Sample* collection or results management, a representative of the *National Olympic Committee*, *National Sport Federation*, *International Federation*, and a translator shall be authorized to attend the "B" confirmation.

In the absence of all of the above persons, the Testing Authority or the Laboratory shall appoint a surrogate (independent witness) to verify that the "B" *Sample* container shows no signs of tampering and that the identifying numbers match that on the collection documentation.

The Laboratory Director may limit the number of individuals in Controlled Zones of the Laboratory based on safety or security considerations.

The Laboratory Director may remove, or have removed by proper authority, any *Athlete* or representative that is interfering in the testing process. Any behavior resulting in removal should be reported to the Testing Authority and may be considered *anti-doping rule violation in accordance with Article 2.5 of the Code, "Tampering, or Attempting to tamper, with any part of Doping Control"*.

5.2.4.3.2.5 Aliquots taken for analysis must be taken from the original "B" *Sample*.

5.2.4.3.2.6 The Laboratory must have a policy to define those circumstances when confirmation testing of the "B" *Sample* may be repeated. Each repeat confirmation should be performed on a new Aliquot of the "B" *Sample*.

5.2.4.3.2.7 If the "B" *Sample* confirmation does not provide analytical findings that confirm the "A" *Sample* result, the *Sample* shall be considered negative and the Testing Authority notified of the new analytical finding.

#### 5.2.4.4 Alternative biological matrices screening and confirmatory testing

5.2.4.4.1 Unless otherwise defined, this application applies only to the analysis of urine *Samples*. Blood, plasma, and serum are acceptable matrices for testing in certain circumstances. Specific requirements for the testing of these matrices are not included in the scope of this document and will be promulgated separately.

5.2.4.4.2 Any testing results of hair, nails, oral fluid or other biological material shall not be used to counter *Adverse Analytical Findings* from urine.

### 5.2.5 Results Management

#### 5.2.5.1 Review of results

5.2.5.1.1 A minimum of two certifying scientists must independently review all *Adverse Analytical Findings* before a report is issued. The review process shall be documented.

5.2.5.1.2 At a minimum, the review shall include:

- Laboratory Internal Chain of Custody documentation
- Urine integrity data
- Validity of the analytical screening and confirmation data and calculations
- Quality control data
- Completeness of documentation supporting the reported analytical findings

5.2.5.1.3 When an *Adverse Analytical Finding* is rejected, the reason(s) must be documented.

### 5.2.6 Documentation and Reporting

5.2.6.1 The Laboratory must have documented procedures to ensure that it maintains a coordinated record related to each *Sample* analyzed. In the case of an *Adverse Analytical Finding*, the record must include the data necessary to support the conclusions reported (as set forth in the Technical Document, Laboratory Documentation Packages). In general, the record should be such that in the absence of the analyst, another competent analyst could evaluate what tests had been performed and interpret the data.

5.2.6.2 Each step of testing shall be traceable to the staff member who performed that step.

5.2.6.3 Significant variance from the written procedure shall be documented as part of the record (e.g., memorandum for the record).

5.2.6.4 Where instrumental analyses are conducted, the operating parameters for each run shall be recorded.

5.2.6.5 Reporting of "A" *Sample* results should occur within ten (10) working days of receipt of the *Sample*. The reporting time required for specific competitions may be substantially less than ten days. The reporting time may be modified by agreement between the Laboratory and the Testing Authority.

5.2.6.6 The Laboratory Certificate of Analysis or Test Report shall include, in addition to the items stipulated in ISO 17025, the following:

- *Sample* identification number
- Laboratory identification number (if any)
- Status of test (*Out of competition/In-competition*)
- Name of competition and/or sport
- Date of receipt of *Sample*
- Date of report
- Type of sample (urine, blood, etc.)
- Test results
- Signature of certifying individual
- Other information as specified by the Testing Authority.

5.2.6.7 The Laboratory is not required to measure or report a concentration for *Prohibited Substances* for a non-threshold analyte. The Laboratory should report the actual *Prohibited Substance(s)*, *Metabolite(s)* of the *Prohibited Substance(s)* or *Method(s)*, or *Marker(s)* detected in the *Sample*.

5.2.6.8 For Threshold Substances, the Laboratory report should establish that the *Prohibited Substance* or its *Metabolite(s)* or *Marker(s)* of a *Prohibited Method* is present at a concentration greater than the threshold concentration taking into consideration the uncertainty in concluding that the concentration in the *Sample* exceeds the threshold. The estimate of uncertainty should not be included on the Certificate of Analysis or Test Report but must be included in Laboratory Documentation Packages.

5.2.6.9 The Laboratory shall have a policy regarding the provision of opinions and interpretation of data. An opinion or interpretation may be included in the Certificate of Analysis or Test Report provided that the opinion or interpretation is clearly identified as such. The basis upon which the opinion has been made shall be documented.

Note: An opinion or interpretation may include, but not be limited to, recommendations on how to use results, information related to the pharmacology, metabolism and pharmacokinetics of a substance, and whether an observed result is consistent with a set of reported conditions.

5.2.6.10 In addition to reporting to the Testing Authority, the Laboratory shall simultaneously report any *Adverse Analytical Findings* to WADA and the responsible International Federation. In the case where the sport or *Event* is not associated with an International Federation (e.g., college sports) or the *Athletes* are not members of an International Federation, the Laboratory is required to report *Adverse Analytical Findings* only to WADA. All reporting shall be in accord with the confidentiality requirements of the *Code*.

5.2.6.11 The Laboratory shall report quarterly to WADA, in a format specified by WADA, a summary of the results of all tests performed. No information that could link an *Athlete* with an individual result will be included. The report will include a summary of any *Samples* rejected for testing and the reason for the rejection.

When the clearinghouse is in place, the Laboratory shall simultaneously report to WADA all information reported to the Testing Authority, according to the requirements listed in Section 5.2.6.6, in lieu of the paragraph above. The information will be used to generate summary reports.

5.2.6.12 Laboratory Documentation Packages shall contain material specified in the WADA Technical Document on Laboratory Documentation Packages.

5.2.6.13 *Athlete* confidentiality is a key concern for all Laboratories engaged in *Doping Control* cases. Confidentiality requires extra safeguards given the sensitive nature of these tests.

5.2.6.13.1 Testing Authority requests for information must be made in writing to the Laboratories.

5.2.6.13.2 *Adverse Analytical Findings* shall not be provided by telephone.

5.2.6.13.3 Information sent by a facsimile is acceptable if the security of the receiving facsimile machine has been verified and procedures are in place to ensure that the facsimile has been transmitted to the correct facsimile number.

5.2.6.13.4 Unencrypted email is not authorized for any reporting or discussion of *Adverse Analytical Findings* if the *Athlete* can be identified or if any information regarding the identity of the *Athlete* is included. The Laboratory shall also provide any information requested by WADA in conjunction with the Monitoring Program, as set forth in Article 4.5 of the *Code*.

### **5.3 Quality Management Processes**

#### **5.3.1 Organization**

5.3.1.1 Within the framework of ISO/IEC 17025, the Laboratory shall be considered a testing laboratory (and not a calibration laboratory).

5.3.1.2 The Laboratory (Scientific) Director shall have the responsibilities of the Chief Executive, unless otherwise noted.

#### **5.3.2 Quality Policy and Objectives**

5.3.2.1 The Quality Policy and implementation shall meet the requirements of ISO/IEC 17025 Section 4.2 Quality Management System and shall include a quality manual that describes the quality system.

5.3.2.2 A single staff member should be appointed as the Quality Manager and should have responsibility and authority to implement and ensure compliance with the quality system.

#### **5.3.3 Document Control**

The control of documents that make up the Quality Management System shall meet the requirements of ISO/IEC 17025 Section 4.3 Document Control

5.3.3.1 The Laboratory Director (or designee) shall approve the Quality Manual and all other documents used by staff members in completing testing.

5.3.3.2 The Quality Management System shall ensure that the contents of WADA Technical Documents are incorporated into the appropriate manuals by the effective date and that training is provided and documented. If this is not possible, WADA should be contacted with a written request for an extension.

#### **5.3.4 Review of requests, tenders, and contracts**

Review of legal documents or agreements related to testing must meet the requirements of ISO/IEC 17025 Section 4.4.

The Laboratory shall ensure that the Testing Authority is informed concerning the tests that can be performed on *Samples* submitted for analysis.

#### **5.3.5 Subcontracting of tests**

A WADA-accredited Laboratory must perform all work with its own personnel and equipment within its accredited facility. In the case of specific technologies that may not be available in the Laboratory (e.g., GC/C/IRMS, Isoelectric focusing [EPQ/NESP]), a *Sample* may be transferred to another WADA-accredited Laboratory in which the technology is within the scope of analysis.



In exceptional circumstances, WADA may elect to grant specific authorization for subcontracting part of the tasks. In such cases, assurance of maintaining the level of quality and the appropriate chain of custody throughout the entire process is the responsibility of the Laboratory Director of the WADA-accredited Laboratory.

### **5.3.6 Purchasing of services and supplies**

#### **5.3.6.1 Chemicals and reagents**

Chemicals and reagents must be suitable for the purpose and be of established purity. Reference purity documentation must be obtained when available and retained in the quality system documents.

In the case of rare or difficult to obtain reagents, Reference Materials, or Reference Collections, particularly for use in qualitative methods, the expiration date of the solution can be extended if adequate documentation exists that no significant deterioration has occurred.

5.3.6.2 Waste disposal shall be in accord with national laws and other relevant regulations. This includes biohazard materials, chemicals, controlled substances, and radioisotopes, if used.

5.3.6.3 Environmental health and safety policies should be in place to protect the staff, the public, and the environment.

### **5.3.7 Service to the client**

5.3.7.1 Service to clients shall be handled in accord with ISO/IEC 17025 Section 4.7.

#### **5.3.7.2 Ensuring responsiveness to WADA**

The Laboratory Director or his designee must:

- Ensure adequate communication.
- Report to WADA any unusual circumstances or information with regard to testing programs; patterns of irregularities in *Specimens*, or potential Use of new substances.
- Provide complete and timely explanatory information to WADA as appropriate and as requested to provide quality accreditation.

#### **5.3.7.3 Ensuring Testing Authority focus**

5.3.7.3.1 The Laboratory Director shall be familiar with the Testing Authority rules and the *Prohibited List*.

5.3.7.3.2 The Laboratory Director should interact with the Testing Authority with respect to specific timing, report information, or other support needs. These interactions should include, but are not limited to, the following:

- Communicate with the Testing Authority concerning any significant question of testing needs or any unusual circumstance in the testing process (including delays in reporting).
- Act without bias regarding the national affiliation of the Testing Authority.
- Provide complete and timely explanations to the Testing Authority when requested or when there is a potential for misunderstanding the Test Report or Certificate of Analysis.
- Provide evidence and/or expert testimony on any test result or report produced by the Laboratory as required in administrative, arbitration, or legal proceedings.
- Respond to any comment or complaint submitted by a Testing Authority or Anti-Doping Organization concerning the Laboratory and its operation.

5.3.7.3.3 The Laboratory shall monitor Testing Authority satisfaction. There should be documentation that the Testing Authority concerns have been incorporated into the Laboratory Quality Management System, where appropriate.

5.3.7.3.4 The Laboratory shall develop a system, as required by ISO 17025, for monitoring key indicators of Laboratory service.

### **5.3.8 Complaints**

Complaints shall be handled in accord with ISO/IEC 17025 Section 4.8.

### **5.3.9 Control of nonconforming testing work**

5.3.9.1 The Laboratory shall have policies and procedures that shall be implemented when any aspect of its testing or a result from its testing does not comply to set procedures.

5.3.9.2 Documentation of any non-compliance or deviation from procedure or protocol involving a *Sample* testing shall be kept as part of the permanent record of that *Sample*.

### **5.3.10 Corrective action**

Corrective action shall be taken in accord with ISO/IEC 17025 Section 4.10.

### **5.3.11 Preventive action**

Preventive action shall be taken in accord with ISO/IEC 17025 Section 4.11.

### **5.3.12 Control of records**

#### **5.3.12.1 Technical Records**

5.3.12.1.1 Analytical records on negative *Samples*, including Laboratory Internal Chain of Custody documentation and medical information (T/E ratio, steroid profiles, and blood parameters), must be

retained in secure storage for at least two (2) years. Relevant records on *Samples* with irregularities or rejected *Samples* must be retained in secure storage for at least two (2) years.

5.3.12.1.2 All analytical records on *Specimens* with an *Adverse Analytical Finding* must be retained in secure storage at least five (5) years, unless otherwise specified by the Testing Authority or by contract.

5.3.12.1.3 The raw data supporting all analytical results must be retained in secure storage for five (5) years.

### 5.3.13 Internal Audits

5.3.13.1 Internal audits shall be completed in accordance with the requirements of ISO/IEC 17025 Section 4.13.

5.3.13.2 Internal Audit responsibilities may be shared amongst personnel provided that any *Person* does not audit his/her own area.

### 5.3.14 Management Reviews

5.3.14.1 Management reviews will be conducted to meet the requirements of ISO/IEC 17025 Section 4.14.

5.3.14.2 WADA will publish, from time to time, specific technical recommendations in a Technical Document. Implementation of the technical recommendations described in the Technical Documents is mandatory and should occur by the effective date.

Technical Documents supersede any previous publication on a similar topic, or if applicable, this document. The document in effect will be that Technical Document whose effective date most recently precedes that of *Sample* receipt date. The current version of the Technical Document will be available on WADA's website.

## 5.4 Support processes

### 5.4.1 General

General support shall be provided in accord with ISO/IEC 17025.

### 5.4.2 Personnel

5.4.2.1 Every person employed by, or under contract to, the Laboratory must have a personnel file accessible for auditors. The file must contain copies of the resumé, or qualification form, a description of the job, and documentation of initial and ongoing training. The Laboratory must maintain appropriate confidentiality of personal information.

5.4.2.2 All personnel should have a thorough knowledge of their responsibilities including the security of the Laboratory, confidentiality of results, Laboratory Internal Chain of Custody protocols, and the standard operating procedures for any method that they perform.

5.4.2.3 The Laboratory Director is responsible for ensuring that Laboratory personnel are adequately trained and have experience necessary to perform their duties. The certification should be documented in the individual's personnel file.

5.4.2.4 The Doping Control Laboratory must have a qualified person as the Laboratory Director to assume professional, organizational, educational, and administrative responsibility. The Laboratory Director qualifications are:

- Ph.D. or equivalent in one of the natural sciences or Training comparable to a Ph.D. in one of the natural sciences such as a medical or scientific degree with appropriate experience or training.
- Experience with the analysis of biological material for substances used in doping.
- Appropriate training or experience in forensic applications of Doping Control.

5.4.2.5 The Doping Control Laboratory must have qualified personnel to serve as Certifying Scientist(s) to review all pertinent data, quality control results, and to attest to the validity of the Laboratory's test reports. The qualifications are:

- Bachelors Degree In Medical Technology, Chemistry, Biology, or related natural science or equivalent. Documented experience of 8 years or more in a Doping Control Laboratory is equivalent to a Bachelor's degree for this position.
- Experience in the analysis of doping materials in biological fluids.
- Experience in the use of relevant analytical techniques such as chromatography, immunoassay, and Gas Chromatography/Mass Spectrometry.

5.4.2.6 Supervisory personnel should have a thorough understanding of the Quality Control procedures; the review, interpretation, and reporting of test results; maintenance of Laboratory Internal Chain of Custody; and proper remedial action to be taken in response to analytical problems. The qualifications for supervisor are:

- Bachelors Degree in Medical Technology, Chemistry, Biology, or related natural science or equivalent. Documented experience of 5 years or more in a Doping Control Laboratory is equivalent to a Bachelor's degree for this position.

- Experience in relevant analytical testing including the analysis of *Prohibited Substances* in biological material.
- Experience in the use of analytical techniques such as chromatography, immunoassay, and Gas Chromatography/Mass Spectrometry.
- Ability to ensure compliance with quality management systems and quality assurance processes.

#### **5.4.3 Accommodation and environmental conditions**

##### **5.4.3.1 Environmental Control**

###### **5.4.3.1.1 Maintain appropriate electrical services**

5.4.3.1.1.1 The Laboratory shall ensure that adequate electrical service is available so that there is no interruption or compromise of stored data.

5.4.3.1.1.2 All computers, peripherals, and communication devices should be supported in such a way that service is not likely to be interrupted.

5.4.3.1.1.3 The Laboratory shall have policies in place to ensure the integrity of refrigerated and/or frozen stored samples in the event of an electrical failure.

5.4.3.1.2 The Laboratory shall have a written safety policy and compliance with Laboratory safety policies shall be enforced.

5.4.3.1.3 The storage and handling of controlled substances must comply with applicable national legislation.

##### **5.4.3.2 Security of the facility**

5.4.3.2.1 The Laboratory shall have a policy for the security of its facilities, which may include a threat and risk assessment.

5.4.3.2.2 Three levels of access should be considered in the quality manual or threat assessment plan:

- Reception zone. An initial point of control beyond which unauthorized individuals must be escorted.
- Common operational zones.
- Controlled zones. Access to these areas should be monitored and records maintained of access by visitors.

5.4.3.2.3 The Laboratory shall restrict access to Controlled Zones to only authorized persons. A staff member should be assigned as the

security officer who has overall knowledge and control of the security system.

5.4.3.2.4 Unauthorized persons must be escorted within Controlled Zones. A temporary authorization may be issued to individuals requiring access to the Controlled Zones such as auditing teams and individuals performing service or repair.

5.4.3.2.5 It is advisable to have a separate Controlled Zone for *Sample* receipt and Aliquot preparation.

#### 5.4.4 Test Methods and Method Validation

##### 5.4.4.1 Selection of Methods

Standard methods are generally not available for *Doping Control* analyses. The Laboratory shall develop, validate, and document in-house methods for compounds present on the *Prohibited List* and for related substances. The methods shall be selected and validated so they are fit for the purpose.

##### 5.4.4.1.1 Non-threshold Substances

Laboratories are not required to measure or report a concentration for Non-threshold Substances.

The Laboratory must develop as part of the method validation process acceptable standards for identification of *Prohibited Substances*. (See the Technical Document on Identification Criteria for Qualitative Assays)

The Laboratory must demonstrate the ability to achieve the Minimum Required Performance Limits using a representative substance or substances if the appropriate standards are available. In case a Reference Collection is used for identification, an estimate of the limit of detection for the method must be provided by assessing a representative substance.

##### 5.4.4.1.2 Threshold Substances

The Laboratory must develop methods with an acceptable uncertainty near the threshold concentration. The method must be capable of documenting both the relative concentration and the identity of the *Prohibited Substance* or *Metabolite(s)* or *Marker(s)*.

Confirmation methods for Threshold Substances must be performed on three Aliquots from the "A" bottle and three Aliquots from the "B" bottle, if the "B" sample confirmation is performed. If insufficient Sample volume exists to analyze three Aliquots, the maximum number of Aliquots that can be prepared should be analyzed. *Adverse Analytical Finding* decisions shall be based on the mean of the measured

concentrations and include consideration of uncertainty with the coverage factor,  $k$ , reflecting the number of Aliquots analyzed and a level of confidence of 95%. Reports and documentation, where necessary, shall report the mean concentration.

#### 5.4.4.1.3 Minimum Required Performance Limit

For both Non-threshold and Threshold Substances, the Laboratory will be required to meet a Minimum Required Performance Limit for detection, identification, and demonstration that a substance exceeds the threshold (if required).

#### 5.4.4.2 Validation of Methods

5.4.4.2.1 Confirmation methods for Non-threshold Substances must be validated. Examples of factors relevant to determining if the method is fit for the purpose are:

- **Specificity.** The ability of the assay to detect only the substance of interest must be determined and documented. The assay must be able to discriminate between compounds of closely related structures.
- **Identification capability.** Since the results for Non-threshold substances are not quantitative, the Laboratory should establish criteria for ensuring that identification of a substance representative of the class of *Prohibited Substances* can be repeatedly identified and detected as present in the sample at a concentration near the MRPL.
- **Robustness.** The method must be determined to produce the same results with respect to minor variations in analytical conditions. Those conditions that are critical to reproducible results must be controlled.
- **Carryover.** The conditions required to eliminate carryover of the substance of interest from sample to sample during processing or instrumental analysis must be determined and implemented.
- **Matrix interferences.** The method should avoid interference in the detection of *Prohibited Substances* or their *Metabolites* or *Markers* by components of the sample matrix.
- **Standards.** Reference standards should be used for identification, if available. If there is no reference standard

available, the use of data or sample from a validated Reference Collection is acceptable.

5.4.4.2.2 Confirmation methods for Threshold Substances must be validated. Examples of factors relevant to determining if the method is fit for the purpose are:

- **Specificity.** The ability of the assay to detect only the substance of interest must be determined and documented. The assay must be able to discriminate between compounds of closely related structures.
- **Intermediate Precision.** The method must allow for the reliable repetition of the results at different times and with different operators performing the assay. Intermediate Precision at the threshold must be documented.
- **Robustness.** The method must be determined to produce the same results with respect to minor variations in analytical conditions. Those conditions that are critical to reproducible results must be controlled.
- **Carryover.** The conditions required to eliminate carryover of the substance of interest from sample to sample during processing or instrumental analysis must be determined and implemented
- **Matrix interferences.** The method must limit interference in the measurement of the amount of *Prohibited Substances* or their *Metabolites* or *Markers* by components of the sample matrix.
- **Standards.** Reference standards should be used for quantification, if available. If there is no reference standard available, the use of data or sample from a validated Reference Collection is acceptable.
- **Minimum Required Performance Limits (MRPL).** The Laboratory must demonstrate that it can detect representative compounds of each prohibited class at defined MRPLs. The Laboratory should also determine the limit of detection and limit of quantification if the MRPL is close to these limits.
- **Linearity** must be documented at 50% to 200% of the threshold value, unless otherwise stipulated in a Technical Document.



#### 5.4.4.3 Estimate of Uncertainty of Method

In most cases an identification of a *Prohibited Substance*, its *Metabolite(s)* or *Marker(s)*, is sufficient to report an *Adverse Analytical Finding*. Thus, quantitative uncertainty as defined in ISO/IEC 17025 does not apply. In the identification of a compound by GC/MS or HPLC/MS, there are qualitative measures that substantially decrease the uncertainty of identification.

In the case of a Threshold Substance, uncertainty in both the identification and the finding that the substance is present in an amount greater than the threshold concentration must be addressed:

##### 5.4.4.3.1 Uncertainty in Identification

The appropriate analytical characteristics must be documented for a particular assay. The Laboratory must establish criteria for identification of a compound at least as strict as those stated in any relevant Technical Document.

##### 5.4.4.3.2 Uncertainty in establishing that a substance exceeds a threshold.

The purpose of threshold reporting in *Doping Control* is to establish that the *Prohibited Substance* or its *Metabolite(s)* or *Marker(s)* are present at a concentration greater than the threshold value. The method, including selection of standards and controls, and report of uncertainty should be designed to fit the purpose.

##### 5.4.4.3.2.1 Uncertainty of quantitative results, particularly at the threshold value, should be addressed during the validation of the assay through measurement of Repeatability, Intermediate Precision and bias, where possible.

##### 5.4.4.3.2.2 The expression of uncertainty should use the expanded uncertainty using a coverage factor, $k$ , to reflect a level of confidence of 95 %. The expression of uncertainty may also take the form of a one-sided t-test at a level of confidence of 95 %.

##### 5.4.4.3.2.3 Uncertainty may be further addressed in Technical Documents in order to reflect the purpose of analysis for the specific substances.

#### 5.4.4.4 Control of Data

##### 5.4.4.4.1 Data and Computer Security

##### 5.4.4.4.1.1 Access to computer terminals, computers, or other operating equipment shall be controlled by physical access and by multiple levels of access controlled by

passwords or other means of employee recognition and identification. These include, but are not limited to account privileges, user identification codes, disk access, and file access control.

5.4.4.4.1.2 The operating software and all files shall be backed up on a regular basis and a current copy kept off site at a secure location.

5.4.4.4.1.3 The software shall prevent the changing of results unless there is a system to document the person doing the editing and that editing can be limited to users with proper level of access.

5.4.4.4.1.4 All data entry, recording of reporting processes and all changes to reported data shall be recorded with an audit trail. This shall include the date and time, the information that was changed, and the individual performing the task.

## **5.4.5 Equipment**

5.4.5.1 A List of available equipment is to be established and maintained.

5.4.5.2 As part of a quality system, the Laboratories shall operate a program for the maintenance and calibration of equipment according to ISO 17025 Section 5.5.

5.4.5.3 General service equipment that is not used for making measurements should be maintained by visual examination, safety checks, and cleaning as necessary. Calibrations are only required where the setting can significantly change the test result. A maintenance schedule shall be established for items such as fume hoods, centrifuges, evaporators, etc, which are used in the test method.

5.4.5.4 Equipment or volumetric devices used in measuring shall have periodic performance checks along with servicing, cleaning, and repair.

5.4.5.5 Qualified subcontracted vendors may be used to service, maintain, and repair measuring equipment.

5.4.5.6 All maintenance, service, and repair of equipment must be documented.

## 5.4.6 Measurement Traceability

### 5.4.6.1 Reference Standards

Few of the available reference drug and drug *Metabolite(s)* are traceable to national or international standards. When available, reference drug or drug *Metabolite(s)* traceable to a national standard or certified by a body of recognized status, such as USP, BP, Ph.Eur. or WHO, should be used. When available, a certificate of analysis or authenticity shall be obtained.

When a reference standard is not certified, the Laboratory shall verify its identity and purity by comparison with published data or by chemical characterization.

### 5.4.6.2 Reference Collections

A collection of samples or isolates may be obtained from a biological matrix following an authentic and verifiable administration of a *Prohibited Substance* or *Method*, providing that the analytical data are sufficient to justify the identity of the relevant chromatographic peak or isolate as a *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method*.

## 5.4.7 Assuring the quality of test results

5.4.7.1 The Laboratory must participate in the WADA Proficiency Testing Program.

5.4.7.2 The Laboratory shall have in place a quality assurance system, including the submission of blind quality control samples, that challenges the entire scope of the testing process (i.e, sample receipt and accessioning through result reporting).

5.4.7.3 Analytical performance should be monitored by operating quality control schemes appropriate to the type and frequency of testing performed by the Laboratory. The range of quality control activities includes:

- Positive and negative controls analyzed in the same analytical run as the *Presumptive Adverse Analytical Finding Sample*.
- The use of deuterated or other internal standards or standard addition.
- Comparison of mass spectra or ion ratios from selected ion monitoring (SIM) to a Reference Material or Reference Collection sample analyzed in the same analytical run
- Confirmation of the "A" and "B" Split Samples.

- Quality control charts using appropriate control limits (e.g.,  $\pm 20\%$  of the target value) depending on the analytical method employed.
- The quality control procedures should be documented in the Laboratory.

## 6.0 Process of WADA Accreditation

This section describes the technical and financial requirements the laboratory must fulfill in the process of being accredited by WADA. The description of the steps in the accreditation process is linked to the defined requirement presented in Section 4.

### 6.1 Applying for a WADA Laboratory Accreditation

#### 6.1.1 Submit Application Form

The laboratory must fill in the necessary information in the Application Form as provided by WADA and deliver this to WADA with the required documentation and applicable fee. The Application shall be signed by the Laboratory Director and, if relevant, by the Director of the host organization.

#### 6.1.2 Description of Laboratory

As preparations for an initial visit by WADA, the laboratory shall complete a questionnaire provided by WADA and submit it to WADA no later than four weeks after the receipt of the questionnaire. The following information shall be submitted through the questionnaire:

- List of staff and their qualifications
- Description of physical facilities, including a description of the security considerations for *Samples* and records
- List of proposed and actual instrumental resources and equipment
- List of available Reference Materials or standards, or plans to acquire Reference Materials or standards, including properly validated biological Sample Reference Collections
- Financial or business plan for the laboratory

WADA may require an update of this documentation during the process of accreditation.

#### 6.1.3 Provide a letter of support

According to 4.1.2 the laboratory shall provide necessary letters of support containing the required information from the relevant national public authorities, or *National Olympic Committee*, or *National Anti-Doping Organization*.

#### 6.1.4 Conduct Initial visit

If necessary, WADA shall conduct an initial visit (2-3 days) to the laboratory at the laboratory's expense. The purpose of this visit is to clarify issues with regard to the accreditation process and the defined requirements in *the International Standard* for

Laboratories and to obtain information about different aspects of the laboratory relevant for the accreditation.

#### **6.1.5 Issue final report and recommendation**

Within eight (8) weeks after the initial visit or the receipt of the questionnaire, WADA will complete and submit a report to the laboratory. In the report WADA will make the necessary recommendations concerning giving the laboratory status as a WADA Probationary laboratory or if this is not the case, identifying needed improvements in order to be a WADA Probationary laboratory.

### **6.2 Preparing for WADA Laboratory Accreditation**

A probationary period shall be defined for a WADA Probationary Laboratory. The period will range from 12 to 24 months depending on the status of the laboratory with regard to the defined requirements (refer to Section 4.1). The main purpose of this period is that the laboratory shall prepare for initial accreditation. During this period, WADA will provide appropriate feedback to assist the laboratory in improving the quality of its testing process. In this period the laboratory shall:

#### **6.2.1 Obtain ISO 17025 accreditation**

The laboratory shall prepare and establish the required documentation and system according to the requirements in Application of ISO 17025 to Analysis of *Doping Control Sample* (Section 5) and the ISO 17025. Based on this, the laboratory shall initiate and prepare for the accreditation process by consulting with a relevant national accreditation body. An audit team consisting of representatives from a national accreditation body, including independent technical assessors recommended by WADA will audit the laboratory. Copies of the Audit Report shall be sent to WADA. The laboratory has to correct any identified non-conformities within defined time-frames and document this accordingly. Copies of the documentation of the correction of the non-conformities should be sent to WADA.

#### **6.2.2 Participate in the WADA Proficiency Testing Program**

The laboratory must complete a minimum of one year of successful participation in the WADA Proficiency Testing program prior to achieving initial accreditation. (See Annex A for description of the Proficiency Testing program.)

As a final proficiency test, the laboratory shall analyze 20-50 urine *Samples* in the presence of a WADA representative. Costs associated with the WADA on-site visit shall be at the laboratory's expense. The laboratory shall successfully identify and/or document a concentration in excess of the threshold of all of the *Prohibited Substances*, *Metabolite(s)* of *Prohibited Substances*, or *Marker(s)* of *Prohibited Substances* or *Methods* within five (5) days of the laboratory opening the *Samples*. The laboratory shall provide a Certificate of Analysis for each of the *Samples* in the proficiency test. For negative *Samples*, WADA may request all or a portion of the negative screening data. For each of the *Samples* for which there is an *Adverse Analytical Finding*, the laboratory shall provide a Laboratory Documentation Package. This data shall be submitted within two (2) weeks of submission of the initial report.

### **6.2.3 Implement Code of Ethics**

The laboratory shall communicate the Code of Ethics (Annex B) to all employees and ensure understanding of and commitment to the different aspects of the Code of Ethics.

### **6.2.4 Plan and implement research activities**

The laboratory shall develop a plan for its research and development activities in the field of *Doping Control* within a 3 year period including a budget. At least two research and development activities shall be initiated and implemented within the probationary period.

### **6.2.5 Plan and implement sharing of knowledge**

The laboratory shall prepare and convey information and knowledge on at least two specific issues to the other WADA accredited Laboratories within the probationary period.

## **6.3 Obtaining WADA Accreditation**

### **6.3.1 Participate in a WADA accreditation audit**

In the last phase of the probationary period WADA will prepare in cooperation with the laboratory a final WADA accreditation audit. Representatives of WADA will audit compliance of the defined requirements in the Application of ISO 17025 to Analysis of *Doping Control Samples* (Section 5) and the practice and documentation of the laboratory. If WADA has participated in the initial ISO audit, the final WADA audit may be a document audit. Otherwise, the audit can be conducted together with the national accreditation body or separately if more practical. Should an on-site audit take place by WADA, the associated cost shall be at the laboratory's expense. Based on the audit, WADA will issue an Audit Report and submit this to the laboratory. If needed, the laboratory will have to correct identified non-compliances within defined time-frames and report these to WADA.

### **6.3.2 WADA report and recommendation**

Based on the relevant documentation from the laboratory, any WADA technical advisor feedback, and the relevant accreditation body (Audit Report), WADA will make a final report including a recommendation concerning the accreditation of the laboratory. The report and recommendation will be submitted to the WADA Executive Committee for approval. In case that the recommendation is that the laboratory should not be accredited, the laboratory will have a maximum of six (6) months to correct and improve specific parts of their operation, at which time a further report will be made by WADA.

### **6.3.3 Issue and publication of Accreditation certificate**

A certificate signed by a duly authorized representative of WADA shall be issued in recognition of an accreditation. Such certificate shall specify the name of the Laboratory and the period for which the certificate is valid. Certificates may be

issued after the effective date, with retroactive effect. A list of accredited Laboratories will be published annually by WADA.

## **6.4 Maintaining WADA Accreditation**

### **6.4.1 Provide a new letter of support**

Letter(s) of Support from a national public authority or *National Olympic Committee* or *National Anti-Doping Organization* responsible for a national *Doping Control* program or an International Federation responsible for an international *Doping Control* program shall be required in years in which there is an ISO 17025 re-accreditation audit.

A letter of support from the host organization renewing its commitment to the Laboratory shall also be required in conjunction with each ISO 17025 re-accreditation audit.

### **6.4.2 Document annual number of tests**

The Laboratory shall periodically report the results of all tests performed to WADA in a specified format. WADA will monitor *Sample* test volume performed by the Laboratory. If the number of *Samples* falls below 1500 per year, WADA Laboratory accreditation will be suspended or revoked in accordance with Section 6.4.8.

### **6.4.3 Flexible Accreditation**

WADA accredited Laboratories may add or modify scientific methods or add analytes to its scope of work without the need for approval by the body that completed the ISO/IEC 17025 accreditation of that Laboratory. Any analytical method or procedure must be properly selected and validated and included in the scope of the Laboratory at the next ISO audit if use is continued.

### **6.4.4 Document Compliance with the WADA Laboratory Code of Ethics**

The Laboratory Director must send a letter of compliance to WADA every year.

The Laboratory may be asked to provide documentation of compliance with the provisions of the Code of Ethics (Annex B).

### **6.4.5 Document implemented research activities**

The Laboratory must supply an annual progress report to WADA documenting research and development results in the field of *Doping Control* and dissemination of the results. The Laboratory should also relate research and development plans for the next year.

### **6.4.6 Document implemented sharing of knowledge**

The Laboratory must supply an annual report sharing of knowledge with all other WADA-accredited Laboratories.

#### **6.4.7 Participate in WADA/ISO periodical audits and the re-accreditation audit**

WADA reserves the right to inspect and audit the Laboratory at any time. The notice of the audit/inspection will be made in writing to the Laboratory Director. In exceptional circumstances, the audit/inspection may be unannounced.

##### **6.4.7.1 WADA/ISO Re-accreditation audit**

The Laboratory must receive ISO/IEC 17025 accreditation including compliance with the Application of ISO 17025 for Analysis of *Doping Control Samples* (Section 5 of this document). The audit team may include a WADA Consultant to augment the auditing team selected by the national accrediting body for the re-accreditation audit.

Copies of the audit summary report as well as the Laboratory responses must be sent to WADA. The Laboratory shall also provide a copy of the ISO 17025 certificate obtained from the national certifying body.

##### **6.4.7.2 ISO Periodical audit**

In years when a periodical ISO/IEC 17025 audit is required, the Laboratory shall provide WADA with a copy of any external audits and evidence of corrective actions for any non-compliance.

#### **6.4.8 WADA report and recommendation**

WADA will annually review Laboratory compliance with the requirements listed in Sections 4 and 5. With the exception of re-accreditation and other required on-site audits, the annual review will consist of a documentation audit. WADA may require documentation from the Laboratory. Failure of the Laboratory to provide information requested in evaluating performance by the specified date shall be considered a refusal to cooperate and result in Suspension or Revocation of accreditation.

WADA will consider the overall performance of the Laboratory in making decisions regarding continued accreditation. Applicant Laboratory performance on aspects of the standards described in Section 5 (such as turn-around times, Documentation Package contents; and feedback from client organizations) may be considered in this auditing.

##### **6.4.8.1 Maintenance of accreditation**

In the event that the Laboratory has maintained satisfactory performance, WADA will recommend to the WADA Executive Committee that the Laboratory be re-accredited.

##### **6.4.8.2 Suspension of accreditation**

Whenever WADA has reason to believe that Suspension may be required and that immediate action is necessary in order to protect the interests of WADA and the Olympic movement, WADA may immediately suspend a Laboratory's accreditation. If necessary, such decision may be taken by the Chairman of the WADA Executive Committee.



Examples of actions that could result in Suspension of accreditation include:

- Suspension of ISO 17025 accreditation;
- failure to take appropriate corrective action after an unsatisfactory performance;
- lack of compliance with any of the requirements or standards listed in *WADA International Standard for Laboratories* (including Annex A. Proficiency Testing);
- failure to cooperate with WADA or the relevant Testing Authority in providing documentation;
- failure to comply with the WADA Laboratory Code of Ethics.

WADA may recommend a Suspension of accreditation at any time based on the results of the Proficiency Testing program.

The period and terms of Suspension shall be proportionate to the seriousness of the non-compliance(s) or lack of performance and the need to ensure accurate and reliable drug testing of *Athletes*. A period of Suspension shall be up to 6 months, during which time any non-compliance must be corrected. If the non-compliance is not corrected during the Suspension period, the Laboratory accreditation will be revoked.

In the case of a non-compliance WADA may suspend the Laboratory from performing analyses for any *Prohibited Substances*. If WADA determines that the non-compliance is limited to a class of *Prohibited Substances*, WADA may limit the suspension to analysis for the class of compounds in which the non-compliance occurred.

#### 6.4.8.3 Revocation of accreditation

The WADA Executive Committee revokes accreditation of any Laboratory accredited under these provisions if WADA determines that Revocation is necessary to ensure the full reliability and accuracy of drug tests and the accurate reporting of test results. Revocation of accreditation may be based on, but not limited to, the following considerations:

- Loss of ISO 17025 accreditation;
- Unsatisfactory performance in analyzing and reporting results of drug tests
- Unsatisfactory participation in performance evaluations or Laboratory on-site audits;
- Failure to take appropriate corrective action following an unsatisfactory performance either in *Testing* or in a proficiency test;
- A material violation of this standard or other condition imposed on the Laboratory by WADA;

- Failure to correct a lack of compliance with any of the requirements or standards listed in *WADA International Standard for Laboratories* (including Annex A. Proficiency Testing) during a Suspension period;
- Failure to cooperate with *WADA* or the relevant Testing Authority during the Suspension phase;
- A serious violation of the Code of Ethics;
- Conviction of any key personnel for any criminal offence committed that is related to the operation of the Laboratory; or
- Any other cause that materially affects the ability of the Laboratory to ensure the full reliability and accuracy of drug tests and the accurate reporting of results.

A Laboratory whose accreditation has been revoked is ineligible to perform testing of *Doping Control Samples* for any Testing Authority.

If a Laboratory whose accreditation has been revoked should seek accreditation, it shall begin the process as a new laboratory as described in Section 4.1, unless there are exceptional circumstances or justifications as determined solely by *WADA*. In the case of exceptional circumstances, *WADA* shall determine what steps shall be followed prior to granting a new accreditation.

## 6.4.9 Notification

### 6.4.9.1 Written Notice

When a Laboratory is suspended or *WADA* seeks to revoke accreditation, *WADA* must immediately serve the Laboratory with written notice of the Suspension or proposed Revocation by facsimile mail, personal service, or registered or certified mail, return receipt requested. This notice shall state the following:

- 1) The reason for Suspension or proposed Revocation;
- 2) The terms of the Suspension or proposed Revocation; and
- 3) The period of Suspension.

### 6.4.9.2 Effective Date

A Suspension is immediately effective. A proposed Revocation is effective 30 calendar days after the date on the written notice or, if review is requested, upon *WADA*'s decision to uphold the proposed Revocation. A Laboratory who has received notice that its accreditation is in the process of being revoked shall be suspended until the Revocation is made final or is rescinded by *WADA*. If *WADA* decides not to uphold the Suspension or proposed Revocation, the Suspension is terminated immediately and any proposed Revocation shall not take place.

#### 6.4.9.3 Public Notice

WADA will immediately notify all relevant national public authorities, *National Anti-Doping Organizations*, *National Olympic Committees*, *International Federations*, and the IOC of the name and address of any Laboratory that has had its accreditation suspended or revoked, and the name of any Laboratory that has had its Suspension lifted.

WADA will provide to any Testing Authority, upon written request, WADA's written decision which upholds or denies the Suspension or proposed Revocation.

#### 6.4.10 Re-accreditation Costs

On an annual basis, WADA will invoice the Laboratory for a portion of the costs associated with the re-accreditation process. The Laboratory shall assume the travel and accommodation expenses of the WADA representative(s) in the event of on-site inspections.

#### 6.4.11 Issue and publication of Accreditation certificate

If maintenance of accreditation is approved, the Laboratory shall receive a certificate signed by a duly authorized representative of WADA issued in recognition of such accreditation. Such certificate shall specify the name of the Laboratory and the period for which the certificate shall be valid. Certificates may be issued after the effective date, with retroactive effect.

### 6.5 Accreditation Requirements for Satellite Facilities for Major Events

In general, the reporting time requirements for a major *Event* require that the Laboratory facility be at the location in proximity to the competition such that *Samples* can be delivered by *Event Doping Control* staff. This may require relocation of an existing Laboratory for a period of time sufficient to validate operations at the satellite facility and perform the testing for the *Event*.

In extraordinary circumstances, *Samples* may be transferred to an existing Laboratory facility. There must be agreement between the *Major Event Organization* and WADA regarding whether testing requirements such as turn-around time and the *Athlete* rights are met for in any eventuality. If the Laboratory is functioning within its regular facility, the requirements stated below with respect to facilities do not apply. The Laboratory will, however, be required to report on staffing, equipment, and *Sample* transport issues.

The Laboratory shall be responsible for providing WADA with regular updates on the progress of the testing facilities.

#### 6.5.1 Participate in an initial WADA/ISO visit/inspection

WADA may visit the Laboratory facility as soon as it is available to determine whether the facility is adequate. Expenses related to such a visit shall be at the Laboratory's expense. Particular emphasis will be placed on the adequacy of security

considerations, the physical layout of the space to ensure that adequate separation of various parts of the Laboratory are maintained, and to provide a preliminary review of other key support elements.

#### **6.5.2 Document ISO/IEC 17025 accreditation of the satellite facility**

At least one month prior to the major *Event*, the Laboratory must provide documentation that the national accrediting body has provided ISO/IEC accreditation for the satellite facility in compliance with the Application of ISO/IEC 17025 to the Analysis of *Doping Control Samples* (Section 5). WADA may require that a WADA consultant be present at the national accrediting body audit of the satellite facility. WADA's expenses associated with such audit, will be at the Laboratory's expense.

#### **6.5.3 Complete a Pre-Event Report on Facilities and Staff**

At least one (1) month prior to the *Event*, the Laboratory must report:

- List of Laboratory staff
- List of staff scientists not normally employed by the Laboratory (if required)
- Training plan for new staff scientists
- List of instrumental resources and equipment
- Procedure manual specific to the satellite facility including analytical methods
- Summary of results management process including criteria for determining positive and negative results
- Methods of reporting test results in a secure manner to the appropriate authorities

Any changes that occur prior to the *Event* should be immediately reported to WADA.

Even if the testing is to be done at the Laboratory's regular facility, the *Pre-Event Report* must be completed, particularly in regard to personnel changes and any additional equipment.

#### **6.5.4 Participate in WADA accreditation audit**

WADA may choose to perform an independent on-site audit or a document audit of the satellite facility. Should an on-site audit take place, WADA expenses related to the audit will be at the Laboratory's expense. This audit may include analysis of a set of proficiency testing samples. The full complement of staff must be in attendance. Particular emphasis will be placed on involvement of new staff members to assess their competence.

#### **6.5.5 Review the reports and correct identified non-conformities**

The Laboratory Director must address and correct any identified non-compliances. The audit report and documentation of the corrective actions must be submitted to WADA.

#### **6.5.6 Issue and publication of a temporary and limited Accreditation certificate**

Based on the documentation provided, WADA shall make a decision regarding accreditation of the Laboratory. In the event that accreditation is awarded, WADA shall issue an accreditation for the period of the *Event* and an appropriate time before and after the actual competition.

#### **6.5.7 Monitoring and assessment during the Event**

WADA may choose at its sole discretion to have an observer in the Laboratory during the *Event*. The Laboratory Director is expected to provide full cooperation to the observer.

WADA, in conjunction with the *Major Event Organization*, will submit double blind proficiency testing samples to the Laboratory.

In the event of a false positive, the Laboratory will immediately cease testing for the class of *Prohibited Substances and Methods*. The Laboratory shall apply corrective actions within 12 hours of notification of the false positive. All *Samples* analyzed prior to the false positive will be re-analyzed for the class of *Prohibited Substances and Methods* for which the non-compliance occurred. The results of the investigation and analysis will be presented to WADA within 24 hours unless otherwise agreed in writing.

In the event of a false negative, the Laboratory will be required to investigate the root cause and apply corrective actions within 24 hours of notification of the false negative result. A representative group of *Samples* in appropriate number to ensure that the risk of false negatives is minimal will be re-analyzed for the class of *Prohibited Substances and Methods* for which the non-compliance occurred. The results of the investigation and analysis will be presented to WADA within 48 hours unless otherwise agreed in writing.

## **7.0 Requirements for supporting an Adverse Analytical Finding in the Adjudication Process**

This section describes the relevant procedures to be followed where an *Athlete* challenges an *Adverse Analytical Finding* in a hearing as provided for by the *Code*.

### **7.1 Laboratory Documentation Package**

In support of any *Adverse Analytical Finding* the Laboratory is required to provide the Laboratory Documentation Package described in detail in the Technical Document on Laboratory Documentation Packages.

The Laboratory is not required to provide any documentation not specifically included in the Laboratory Documentation Package. Therefore, the Laboratory is not required to support an *Adverse Analytical Finding* by producing, either to the Testing Authority

or in response to discovery requests related to the hearing, standard operating procedures, general quality management documents (e.g., ISO compliance documents) or any other documents not specifically required by Technical Document on Laboratory Documentation Packages. References in the *International Standard for Laboratories* to ISO requirements are for general quality control purposes only and have no applicability to any adjudication of any specific *Adverse Analytical Finding*.

## **PART THREE: ANNEXES**

### **ANNEX A - WADA PROFICIENCY TESTING PROGRAM**

The WADA Proficiency Testing (PT) Program is designed to evaluate Laboratory proficiency and to improve test result uniformity between Laboratories, and to provide educational opportunities for the WADA-accredited Laboratories. The purpose of the individual PT sample will determine its composition and form.

#### **1. Probationary period**

The Proficiency Testing (PT) program is a part of the initial evaluation of a Laboratory seeking accreditation. In addition to providing samples as part of quarterly PT samples, the WADA will provide upon request samples from past PT rounds in order to allow the applicant Laboratory with an opportunity to evaluate its performance against the recorded performance of accredited Laboratories.

All procedures associated with the handling and testing of the PT samples by the Laboratory are, to the greatest extent possible, to be carried out in a manner identical to that applied to routine Laboratory Samples, unless otherwise specified. No effort should be made to optimize instrument (e.g., change multipliers or chromatographic columns) or method performance prior to analyzing the PT samples unless it is a scheduled maintenance activity. Methods or procedures used in routine testing should be employed.

Successful participation in 12-24 months of PT sample rounds is required before a Laboratory is eligible to be considered for accreditation. The PT samples shall occur at least quarterly and will consist of a minimum of five (5) samples per challenge. At least four (4) PT samples will contain Threshold Substances. Blank and adulterated samples may also be included.

#### **2. Maintenance/Re-accreditation period**

After accreditation, Laboratories shall be challenged with at least five (5) PT samples each quarter. Each year at least two (2) samples will contain Threshold Substances. Blank and adulterated samples may be included.

All procedures associated with the handling and testing of the PT samples by the Laboratory are, to the greatest extent possible, to be carried out in a manner identical to that applied to routine Laboratory Samples, unless otherwise specified. No effort should be made to optimize instrument (e.g., change multipliers or chromatographic columns) or method performance prior to analyzing the PT samples unless it is a scheduled maintenance activity. Methods or procedures not used in routine testing should not be employed.

### 2.1 Open PT Samples

The Laboratory may be directed to analyze a PT sample for a specific *Prohibited Substance*. In general, this approach is used for educational purposes or for data gathering.

### 2.2 Blind PT Samples

The Laboratory will be aware that the sample is a PT sample, but will not be aware of the content of the sample. Performance on blind PT samples is to be at the same level as for the open or non-blind PT samples.

### 2.3 Reporting – Open and Blind Proficiency Samples

The Laboratory should report the results of open and blind PT samples to WADA in the same manner as specified for routine *Samples*. For some samples or PT sample sets, additional information may be requested from the Laboratory.

### 2.4 Double Blind Proficiency Sample

The Laboratory will receive PT sample sets which are indistinguishable from normal testing samples. The samples may consist of blank, adulterated or positive samples. These samples may be used to assess turn-around time, compliance with documentation package requirements, and other non-analytical performance criteria as well as Laboratory proficiency.

## 3. Proficiency Test Sample Composition

### 3.1 Description of the Drugs

PT samples contain those *Prohibited Substances*, *Metabolite(s)* of *Prohibited Substances*, and *Marker(s)* of *Prohibited Substances and Methods* which each accredited Laboratory must be prepared to assay in concentrations that allow detection of the analytes by commonly used screening techniques. These are generally concentrations that might be expected in the urine of drug users. For some analytes, the sample composition may consist of the parent drug as well as major *Metabolites*. The actual composition of the PT samples supplied to different Laboratories in a particular PT sample may vary but, within any annual period, all Laboratories participating are expected to have analyzed the same total set of samples.

A sample may contain more than one *Prohibited Substance*, *Metabolite(s)*, or *Marker* of a *Prohibited Substance or Method*. A PT sample will not contain more than three substances or their *Metabolite(s)*, or *Markers* of *Prohibited Substances or Methods*. It is possible that the sample will contain multiple *Metabolites* of a single substance, which would represent the presence of a single *Prohibited Substance*. All *Metabolites* detected should be reported according to the Laboratory's standard operating procedures.

### 3.2 Concentrations

PT samples may be spiked with *Prohibited Substances* and/or their *Metabolites* or may be from authentic administration studies. For Threshold Substances, the



concentration in the sample will be guided by, but not limited to, one of the following criteria:

- i) at least 20 percent above the threshold for either the initial assay or the confirmatory test, depending on which is to be evaluated;
- ii) near or below the threshold limit for special purposes. In this case, the Laboratory would be directed to analyze the *Sample* for a particular *Prohibited Substance* as part of an educational challenge and will not be considered for evaluation for the purposes of the PT program.

For Non-threshold Substances, the concentration will be guided by, but not limited to, one of the following criteria:

- i) the *Prohibited Substance* and/or its major *Metabolite(s)* will be present in quantities greater than the Minimum Required Performance Limit;
- ii) the *Prohibited Substance* and/or its major *Metabolite(s)* will be present near the limit of detection for special purposes. In this case, the Laboratory would be directed to analyze the sample for a particular *Prohibited Substance* as part of an educational challenge and will not be considered for evaluation for the purposes of the PT program.

These concentrations and drug types may be changed periodically in response to factors such as changes in detection technology and patterns of drug use.

Negative samples do not contain concentrations of any of the target drugs above the Minimum Required Performance Limit when analyzed by the normally used methods.

### 3.3 Blank or Adulterated Samples

PT samples include those that do not contain prohibited drugs or samples which have been deliberately adulterated by the addition of extraneous substances designed to dilute the sample, degrade the analyte or to mask the analyte during the analytical determination.

## 4. Evaluation of Proficiency Testing Results

### 4.1 Evaluation of Quantitative Results

When a quantitative determination has been reported, the results can be scored based on the true or consensus value of the sample analyzed and a standard deviation which may be set either by the group results or according to the expected precision of the measurement. The z-score is calculated using the equation

$$z = \frac{\bar{x} - \hat{x}}{\delta}$$

Where  $x$  is the value found

$\hat{x}$  is the assigned value

$\delta$  is the target value for standard deviation

The target relative standard deviation will be set in such a way that an absolute z-score between two (2) and three (3) is deemed **questionable** performance. A z-score greater than three (3) is deemed **unacceptable** performance.

In addition, re-scaled sum of score (RSZ) and re-scaled sum of squared scores (RSSZ) will be calculated. While the z score gives an estimate of bias, the RSZ, by retaining the sign of the biases, will reflect consistent systematic bias. The RSSZ, by eliminating the possibility that positive and negative bias will cancel, provides another indicator of bias. The RSZ and RSSZ are calculated by the equations

$$RSZ = \sum \frac{z}{\sqrt{m}}$$

$$RSSZ = \sum \frac{z^2}{m}$$

where m is the number of tests.

## 4.2 Probationary Period

**4.2.1** Any false positive reported automatically disqualifies a Laboratory from further consideration for accreditation. The Laboratory will be eligible for reinstatement upon providing documentation that satisfies WADA that remedial and preventative actions have been implemented.

**4.2.2** An applicant Laboratory is to achieve an overall grade level of 90 percent for PT samples required during the probationary period, i.e., it must correctly identify and confirm 90 percent of the total drug challenges (qualitative including adulterated samples).

**4.2.3** An applicant Laboratory is to obtain satisfactory Z-scores for any quantitative results reported based on the mean of three replicate determinations. For the purposes of accreditation a quantitative result is required for threshold drugs. The relative standard deviation is to be commensurate with the validation data.

Any Laboratory that fails to achieve a satisfactory score for at least 90% of the quantitative determinations during the probationary period will be disqualified from further consideration. If the Laboratory receives fewer than 10 samples for quantitation in the year, the Laboratory may be allowed a single unsatisfactory result in the quantitative portion of the PT program during a 12 month period. The Laboratory will be eligible for reinstatement upon providing documentation that satisfies WADA that remedial and preventative actions have been implemented.

### 4.3 Maintenance and Re-Accreditation Period

**4.3.1** No false positive drug identification is acceptable for any drug and the following procedures are to be followed when dealing with such a situation:

- i) The Laboratory is immediately informed of a false positive error by the WADA.
- ii) The Laboratory is to provide the WADA with a written explanation of the reasons for the error within five (5) working days. This explanation is to include the submission of all quality control data from the batch of samples that included the false positive sample. If the error is deemed to be technical/scientific.
- iii) The WADA shall review the Laboratory's explanation promptly and decide what further action, if any, to take.
- iv) If the error is determined to be an administrative error (clerical, sample mix-up, etc), the WADA may direct the Laboratory to take corrective action to minimize the occurrence of the particular error in the future and, if there is reason to believe the error could have been systematic, may require the Laboratory to review and re-analyze previously run *Samples*.
- v) If the error is determined to be a technical or methodological error, the Laboratory may be required to re-test all *Samples* analyzed positive by the Laboratory from the time of final resolution of the error back to the time of the last satisfactory proficiency test round. A statement signed by the Laboratory Director shall document this re-testing. The Laboratory may also be required to notify all clients whose results may have been affected of the error as part of its quality management system. Depending on the type of error that caused the false positive, this retesting may be limited to one analyte, a class of *Prohibited Substances or Methods*, or may include any prohibited drug. The Laboratory shall immediately notify the WADA if any result on a *Sample* that has been reported to a client is detected as a false positive. WADA may suspend or revoke the Laboratory's accreditation. However, if the case is one of a less serious error for which effective corrections have already been made, thus reasonably assuring that the error will not occur again, the WADA may decide to take no further action.
- vi) During the time required to resolve the error, the Laboratory remains accredited but has a designation indicating that a false positive result is pending resolution. If the WADA determines that the Laboratory's accreditation must be suspended or revoked, the Laboratory's official status becomes "Suspended" or "Revoked" until the Suspension or Revocation is lifted or any process complete.

**4.3.2** An accredited Laboratory must correctly identify 100 percent of the *Prohibited Substances* to pass the round of PT samples. It must correctly identify and confirm 100 percent of the total PT samples (qualitative including adulterated samples).

**4.3.3** An accredited Laboratory is to obtain satisfactory Z-scores for any quantitative results reported based on the mean of three replicate determinations. For the purposes of accreditation a quantitative result is required for threshold drugs.

The relative standard deviation is to be commensurate with the validation data.

Any Laboratory that fails to achieve a satisfactory score for quantitative determinations will be deemed to have failed that sample challenge. The Laboratory must achieve a satisfactory score on 90% of the quantitative samples during the year. If the Laboratory receives fewer than 10 samples for quantitation in the year, the Laboratory may be allowed a single unsatisfactory result in the quantitative portion of the PT program during a 12 month period.

**4.4** Laboratories failing a proficiency test round are informed immediately by WADA. Laboratories must take and report corrective action within 30 calendar days to WADA. Laboratories may otherwise be advised by WADA to take corrective action for a given reason or to change a corrective action which has previously been reported to WADA. The corrective action reported to WADA must be implemented in the routine operation of the Laboratory. Repeated failures of the same type will result in WADA requiring corrective action.

Laboratories failing two consecutive rounds of the PT scheme will be immediately suspended. The Laboratory is required to provide documentation of corrective action with 10 working days of notification of Suspension. Failure to do so will result in immediate Revocation of the accreditation. Lifting of the Suspension occurs only when corrective action has been taken and reported to the WADA. The WADA may choose, at its sole discretion, to submit additional PT samples to the Laboratory or to require that the Laboratory be re-audited, at the expense of the Laboratory after having furnished satisfactory results for another proficiency testing round.

**4.5** WADA is to evaluate the annual performance of all accredited Laboratories.

## ANNEX B - LABORATORY CODE OF ETHICS

### 1. Confidentiality

The heads of Laboratories, their delegates and Laboratory staff shall not discuss or comment to the media on individual results prior to the completion of any adjudication without consent of the organization that supplied sample to the Laboratory and the organization that is asserting the *Adverse Analytical Finding* in adjudication.

### 2. Research

Laboratories are entitled to participate in research programs provided that the Laboratory director is satisfied with the *bona fide* nature and the programs have received proper ethical (e.g. human subjects) approval.

#### 2.1. Research in Support of *Doping Control*

The Laboratories are expected to develop a program of research and development to support the scientific foundation of *Doping Control*. This research may consist of the development of new methods or technologies, the pharmacological characterization of a new doping agent, the characterization of a masking agent or method, and other topics relevant to the field of *Doping Control*.

#### 2.2. Human subjects

The Laboratories must follow the Helsinki Accords and any applicable national standards as they relate to the involvement of human subjects in research.

Voluntary informed consent must also be obtained from human subjects in any drug administration studies for the purpose of development of a Reference Collection or proficiency testing materials.

#### 2.3. Controlled substances

The Laboratories are expected to comply with the relevant national laws regarding the handling and storage of controlled (illegal) substances.

### 3. Testing

#### 3.1. Competitions

The Laboratories shall only accept and analyze *Samples* originating from known sources within the context of *Doping Control* programs conducted in competitions organized by national and international sports governing bodies. This includes national and international federations, *National Olympic Committees*, national associations, universities, and other similar organizations. This rule applies to Olympic and non-Olympic sports.

Laboratories should exercise due diligence to ascertain that the *samples* are collected according to the World Anti-Doping *Code International Standard* for

Testing or the International Standard for Doping Control (ISO/PAS 18873), or similar guidelines. These guidelines must include collection of Split Samples; appropriate *Sample* container security considerations; and formal chain of custody conditions.

### **3.2. Out-of-competition**

The Laboratories shall accept *Samples* taken during training (or *Out-of-competition*) only if the following conditions are simultaneously met:

- (a) That the *Samples* have been collected and sealed under the conditions generally prevailing in competitions themselves as in Section 3.1 above;
- (b) If the collection is a part of an anti-doping program; and
- (c) If appropriate sanctions will follow a positive case.

Laboratories shall not accept *Samples*, for the purposes of either screening or identification, from commercial or other sources when the conditions in the above paragraph are not simultaneously met.

Laboratories shall not accept *Samples* from individual *Athletes* on a private basis or from individuals or organizations acting on their behalf.

These rules apply to Olympic and non-Olympic sports.

### **3.3. Clinical or Forensic**

Occasionally the Laboratory is requested to analyze a *Sample* for a banned drug or endogenous substance allegedly coming from a hospitalized or ill *Person* in order to assist a physician in the diagnostic process. Under this circumstance, the Laboratory director must explain the pre-testing issue to the requester and agree subsequently to analyze the *Sample* only if a letter accompanies the *Sample* and explicitly certifies that the *Sample* is for medical diagnostic or therapeutic purposes.

The letter must also explain the medical reason for the test.

Work to aid in forensic investigations may be undertaken but due diligence should be exercised to ensure that the work is requested by an appropriate agency or body. The Laboratory should not engage in testing or expert testimony that would call into question the integrity of the individual or the scientific validity of work performed in the anti-doping program.

### **3.4. Other Testing**

If the Laboratory accepts *Samples* from an entity that is not a Testing Authority recognized by the World Anti-Doping Code, it is the responsibility of the Laboratory Director to ensure that any *Adverse Analytical Finding* will be processed according to the Code and that the results cannot be used in any way by an *Athlete* or associated *Person* to avoid detection.

The Laboratory should not engage in testing that undermines or is detrimental to the anti-doping program of WADA. The Laboratory should not provide results that in any way suggests endorsement of products or services for *Athletes* or sports authorities. The Laboratory should not provide testing services in defense of an *Athlete* in a *Doping Control* adjudication.

### **3.5. Sharing of Information and Resources**

#### **3.6. New Substances**

The WADA-accredited Laboratories for *Doping Control* shall inform WADA when they detect a new or suspicious doping agent.

When possible, the Laboratories shall share information regarding the detection of potentially new or rarely detected doping agents.

#### **3.7. Sharing of Knowledge**

Sharing of knowledge shall consist of, but not be limited to, dissemination of information about new *Prohibited Substances and Methods* and their detection within sixty (60) days of discovery. This can occur by participation in scientific meetings, publication of results of research, sharing of specific details of methodology necessary for detection, and working with WADA to distribute information by preparation of a reference substance or biological excretion study or information regarding the chromatographic retention behaviour and mass spectra of the substance or its *Metabolites*. The Laboratory director or staff shall participate in developing standards for best practice and enhancing uniformity of testing in the WADA-accredited Laboratory system. An example of the latter would be in establishing reporting standards for determination of an *Adverse Analytical Finding*.

### **4. Conduct Detrimental to the Anti-Doping Program**

The Laboratory personnel shall not engage in conduct or activities that undermine or are detrimental to the anti-doping program of WADA, an International Federation, a *National Anti-Doping Organization*, a *National Olympic Committee*, a *Major Event Organization Committee*, or the International Olympic Committee. Such conduct could include, but is not limited to, conviction for fraud, embezzlement, perjury, etc. that would cast doubt on the integrity of the anti-doping program.

No Laboratory employee or consultant shall provide counsel, advice or information to *Athletes* or others regarding techniques or methods to mask detection of, alter metabolism of, or suppress excretion of a *Prohibited Substance or Marker of a Prohibited Substance* or Method in order to avoid an *Adverse Analytical Finding*. No Laboratory staff shall assist an *Athlete* in avoiding collection of a *Sample*. This paragraph does not prohibit presentations to educate *Athletes*, students, or others concerning anti-doping programs and *Prohibited Substances or Methods*.

## ANNEX C - LIST OF TECHNICAL DOCUMENTS

Title	Document Number	Version Number	Effective Date
Laboratory Internal Chain of Custody	TD2003LCOC	1.2	Jan 1, 2004
Laboratory Documentation Packages	TD2003LDOC	1.3	Jan 1, 2004
Minimum Required Performance Limits for Detection of Prohibited Substances	TD2003MRPL	1.2	Jan 1, 2004
Identification Criteria for Qualitative Assays Incorporating Chromatography and Mass Spectrometry	TD2003IDCR	1.2	Jan 1, 2004
Reporting Low Concentrations of Norandrosterone and Noretiochanolone			<i>In progress</i>
Reporting guidance for testosterone, epitestosterone, and other endogenous steroids			<i>In progress</i>
Reporting guidance for Salbutamol and other beta-2 agonists			<i>In progress</i>
Reporting guidance for gas chromatography/combustion/ isotope ratio mass spectrometry			<i>Future</i>
Reporting guidance for recombinant erythropoietin			<i>Future</i>





# The World Anti-Doping Code

## **INTERNATIONAL STANDARD FOR LABORATORIES**

**Version 4.0**

**August 2004**

## PREAMBLE

The World Anti-Doping Code *International Standard for Laboratories* is a mandatory level 2 *International Standard* developed as part of the World Anti-Doping Program.

The basis for the *International Standard for Laboratories* is the relevant Sections in the Olympic Movement Anti-Doping Code. An expert group, together with a WADA *Laboratory Accreditation Committee*, has prepared the document and drafts have been circulated for initial review and comment from all IOC accredited doping *Laboratories* and the IOC Sub-Commission on Doping and Biochemistry of Sport.

Version 1.0 of the *International Standard for Laboratories* was circulated to *Signatories*, governments and accredited laboratories for review and comments in November 2002. Version 2.0 was based on the comments and proposals received from these stakeholders.

All *Signatories*, governments and *Laboratories* were consulted and have had the opportunity to review and provide comments to version 2.0. This draft version 3.0 was presented for approval to the WADA Executive Committee on June 7<sup>th</sup> 2003.

The *International Standard for Laboratories* will come into effect on January 1<sup>st</sup> 2004.

Currently, *Laboratories* are accredited by the International Olympic Committee (IOC). As part of the transition of the program from existing IOC accreditation to WADA accreditation, accreditation bodies shall require the *Laboratories* to which they grant and maintain accreditation to comply with the requirements of the *International Standard for Laboratories* and ISO/IEC 17025 by January 1<sup>st</sup> 2004. For *Laboratories* moving from IOC to WADA accreditation (see Section 4.1.7), an internal audit before January 1<sup>st</sup>, 2004 shall be deemed compliant with the *International Standard for Laboratories*. The next ISO surveillance or re-accreditation audit conducted by the national accrediting body in 2004 shall document compliance with the *International Standard for Laboratories*. *Laboratories* seeking initial WADA accreditation shall have an on-site accreditation audit by their national accrediting body compliant with this standard before receiving WADA accreditation.

The official text of the *International Standard for Laboratories* shall be maintained by WADA and shall be published in English and French. In the event of any conflict between the English and French versions, the English version shall prevail.

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## PART ONE: INTRODUCTION, *CODE* PROVISIONS AND DEFINITIONS

### 1.0 Introduction, Scope and References

The main purpose of the *International Standard for Laboratories* is to ensure laboratory production of valid test results and evidentiary data and to achieve uniform and harmonized results and reporting from all accredited *Doping Control Laboratories*.

The *International Standard for Laboratories* includes requirements for WADA accreditation of doping laboratories, operating standards for laboratory performance and description of the accreditation process.

The *International Standard for Laboratories*, including all Annexes and Technical Documents, is mandatory for all *Signatories* to the *Code*.

The World Anti-Doping Program encompasses all of the elements needed in order to ensure optimal harmonization and best practice in international and national anti-doping programs. The main elements are: the *Code* (Level 1), *International Standards* (Level 2), and Models of Best Practice (Level 3).

In the introduction to the World Anti-Doping Code (*Code*), the purpose and implementation of the *International Standards* are summarized as follows:

"*International Standards* for different technical and operational areas within the anti-doping program will be developed in consultation with the *Signatories* and governments and approved by WADA. The purpose of the *International Standards* is harmonization among *Anti-Doping Organizations* responsible for specific technical and operational parts of the anti-doping programs. Adherence to the *International Standards* is mandatory for compliance with the *Code*. The *International Standards* may be revised from time to time by the WADA Executive Committee after reasonable consultation with the *Signatories* and governments. Unless provided otherwise in the *Code*, *International Standards* and all revisions shall become effective on the date specified in the *International Standard* or revision."

Compliance with an *International Standard* (as opposed to another alternative standard, practice or procedure) shall be sufficient to conclude that the procedures covered by the *International Standard* were performed properly.

This document sets out the requirements for *Doping Control Laboratories* that wish to demonstrate that they are technically competent, operate an effective quality management system, and are able to produce forensically valid results. *Doping Control Testing* involves the detection, identification, and in some cases demonstration of the presence greater than a threshold concentration of drugs and other substances deemed to be prohibited by the list of *Prohibited Substances* and *Prohibited Methods* (*The Prohibited List*) in human biological fluids or tissues.

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The Laboratory accreditation framework consists of two main elements: Part Two of the standard: the Laboratory accreditation requirements and operating standards; and Part Three: the Annexes and Technical Documents. Part Two describes the requirements necessary to obtain WADA recognition and the procedures involved to fulfill the requirements. It also contains an application of the ISO/IEC 17025 standard to the field of *Doping Control*. The purpose of this section of the document is to facilitate consistent application and assessment of the ISO/IEC 17025 and the specific WADA requirements for *Doping Control* by accreditation bodies that operate in accordance with ISO/IEC Guide 58. The *International Standard* also sets forth the requirements for Doping Control Laboratories when adjudication results as a consequence of an *Adverse Analytical Finding*.

Part Three of the Standard includes all Annexes. Annex A describes the WADA Proficiency Testing Program, including performance criteria necessary to maintain good standing in proficiency testing. Annex B describes the ethical standards required for continued WADA recognition of the Laboratory. Annex C is a list of Technical Documents. Technical Documents are issued, modified, and deleted by WADA from time to time and provide direction to the Laboratories on specific technical issues. Once promulgated, Technical Documents become part of the *International Standard for Laboratories*. The incorporation of the provisions of the Technical Documents into the Laboratory's quality management system is mandatory for WADA accreditation.

In order to harmonize the accreditation of Laboratories to the requirements of ISO/IEC 17025 and the WADA-specific requirements for recognition, it is expected that national accreditation bodies will use this standard, including the annexes, as a reference document in their accreditation audit process.

Terms defined in the *Code*, which are included in this standard, are written in italics. Terms, which are defined in this standard, are underlined.

## References

These following references were consulted in the development of this document. The specific requirements and concepts of these documents do not supersede or otherwise change the requirements stated in the *International Standard for Laboratories*.

A2LA, 2001. Proficiency Testing Requirement for Accredited Testing and Calibration Laboratories.

EA-03/04 (August 2001). Use of Proficiency Testing as a Tool for Accreditation in Testing

Eurachem Proficiency Testing Mirror Group (2000). Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories.

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Eurachem/CITAC Guide, 2<sup>nd</sup> Edition (2000) Quantifying Uncertainty in Analytical Measurement.

European Union Decision 2002/657/EC Official Journal of the European Communities 17.8.2002; L 221: 8-36.

ISO/IEC 17025:1999. General requirements for the competence of testing and calibration laboratories.

International Laboratory Accreditation Cooperation (ILAC) Document G-7:1996. Accreditation Requirements and Operating Criteria for Horseracing Laboratories.

ILAC Document G-15:2001. Guidance for Accreditation to ISO/IEC 17025

ILAC Document G-17:2002. Introducing the Concept of Uncertainty of Measurement in Testing in Association with the Application of the Standard ISO/IEC 17025.

ILAC Document G-19:2002. Guideline for Forensic Science Laboratories

ILAC Document P-10:2002. ILAC Policy on Traceability of Measurement Results.

National Clinical Chemistry Laboratory Standards Document C-43A, 2002 [ISBN 1-56238-475-9]. "Gas Chromatography/Mass Spectrometry (GC/MS) Confirmation of Drugs; Approved Guideline."

Olympic Movement Anti-Doping Code (1999)

Society of Forensic Toxicology and American Academy of Forensic Sciences, Toxicology Section, 2002 (Draft). Forensic Toxicology Laboratory Guidelines.

Substance Abuse and Mental Health Services Administration (SAMHSA), United States Department of Health and Human Services (DHHS), 2001. Mandatory Guidelines for Federal Workplace Drug Testing Programs and Notice of Proposed Revisions (Federal Register 2001; 66: 43876-43882).

World Anti-Doping Code.

## 2.0 Code Provisions

The following articles in the *Code* directly address the *International Standard for Laboratories*:

### Code Article 3.2 Methods of Establishing Facts and Presumptions

3.2.1 *WADA*-accredited Laboratories are presumed to have conducted *Sample* analysis and custodial procedures in accordance with the *International Standard* for laboratory analysis. The *Athlete* may rebut this presumption by establishing that a departure from the *International Standard* occurred. If the *Athlete* rebuts the preceding presumption by showing that a departure from the *International Standard* occurred, then the *Anti-Doping Organization* shall have the burden to establish that such departure did not cause the *Adverse Analytical Finding*.

### Code Article 6 Analysis of Samples

*Doping Control Samples* shall be analyzed in accordance with the following principles:

6.1 **Use of Approved Laboratories** *Doping Control Samples* shall be analyzed only in *WADA*-accredited laboratories or as otherwise approved by *WADA*. The choice of the *WADA*-accredited laboratory (or other method approved by *WADA*) used for the *Sample* analysis shall be determined exclusively by the *Anti-Doping Organization* responsible for results management.

[Comment: The phrase "or other method approved by *WADA*" is intended to cover, for example, mobile blood Testing procedures which *WADA* has reviewed and considers to be reliable.]

6.2 **Substances Subject to Detection.** *Doping Control Samples* shall be analyzed to detect *Prohibited Substances* and *Prohibited Methods* identified on the *Prohibited List* and other substances as may be directed by *WADA* pursuant to Article 4.5 (Monitoring Program).

6.3 **Research on Samples.** No *Sample* may be used for any purpose other than the detection of substances (or classes of substances) or methods on the *Prohibited List*, or as otherwise identified by *WADA* pursuant to Article 4.5 (Monitoring Program), without the *Athlete's* written consent.

6.4 **Standards for Sample Analysis and Reporting.** Laboratories shall analyze *Doping Control Samples* and report results in conformity with the *International Standard* for Laboratories analysis.

**Code Article 13.5 Appeals from Decisions Suspending or Revoking Laboratory Accreditation** Decisions by *WADA* to suspend or revoke a Laboratory's *WADA* accreditation may be appealed only by that Laboratory with the appeal being exclusively to CAS.

**Code Article 14.1 Information Concerning Adverse Analytical Findings and Other Potential Anti-Doping Rule Violations.** An *Athlete* whose *Sample* has resulted in an *Adverse Analytical Finding*, or an *Athlete* or other *Person* who may have violated an anti-doping rule, shall be notified by the *Anti-Doping Organization* with results management responsibility as provided in Article 7 (Results Management). The *Athlete's* *National Anti-Doping Organization* and *International Federation* and *WADA* shall also be notified not later than the completion of the process described in Articles 7.1 and 7.2. Notification shall include: the *Athlete's* name, country, sport and discipline within the sport, whether the test was *In-Competition* or *Out-of-Competition*, the date of *Sample* collection and the analytical result reported by the laboratory. The same *Persons* and *Anti-Doping Organizations* shall be regularly updated on the status and findings of any review or proceedings conducted pursuant to Articles 7 (Results Management), 8 (Right to a Fair Hearing) or 13 (Appeals), and, in any case in which the period of *Ineligibility* is eliminated under Article 10.5.1 (*No Fault or Negligence*), or reduced under Article 10.5.2 (*No Significant Fault or Negligence*), shall be provided with a written reasoned decision explaining the basis for the elimination or reduction. The recipient organizations shall not disclose this information beyond those *Persons* within the organization with a need to know until the *Anti-Doping Organization* with

results management responsibility has made public disclosure or has failed to make public disclosure as required in Article 14.2.

## 3.0 Terms and definitions

### 3.1 Code defined Terms

**Adverse Analytical Finding:** A report from a Laboratory or other approved Testing entity that identifies in a Specimen the presence of a Prohibited Substance or its Metabolites or Markers (including elevated quantities of endogenous substances) or evidence of the Use of a Prohibited Method.

**Anti-Doping Organization:** A Signatory that is responsible for adopting rules for, initiating, implementing or enforcing any part of the Doping Control process. This includes, for example, the International Olympic Committee, the International Paralympic Committee, Major Event Organizations that conduct Testing at their Events, WADA, International Federations, and National Anti-Doping Organizations.

**Athlete:** For purposes of Doping Control, any Person who participates in sport at the International level (as defined by each International Federation) or national level (as defined by each National Anti-Doping Organization) and any additional Person who participates in sport at a lower level if designated by the Person's National Anti-Doping Organization. For purposes of anti-doping information and education, any Person who participates in sport under the authority of any Signatory, government, or other sports organization accepting the Code.

**Code:** The World Anti-Doping Code.

**Doping Control:** The process including test distribution planning, Sample collection and handling, Laboratory analysis, results management, hearings and appeals.

**Event:** A series of individual Competitions conducted together under one ruling body (e.g., the Olympic Games, FINA World Championships, or Pan American Games).

**In-competition:** For purposes of differentiating between In-competition and Out-of-Competition Testing, unless provided otherwise in the rules of an International Federation or other relevant Anti-Doping Organization, an In-Competition test is a test where an Athlete is drawn for Testing in connection with a specific Competition.

**International Standard:** A standard adopted by WADA in support of the Code. Compliance with an International Standard (as opposed to another alternative standard, practice or procedure) shall be sufficient to conclude that the procedures covered by the International Standard were performed properly.

**Marker:** A compound, group of compounds or biological parameters that indicates the Use of a Prohibited Substance or Prohibited Method.



**Metabolite:** Any substance produced by a biotransformation process.

**National Anti-Doping Organization:** The entity(ies) designated by each country as possessing the primary authority and responsibility to adopt and implement anti-doping rules, direct the collection of *Samples*, the management of test results, and the conduct of hearings, all at the national level. If this designation has not been made by the competent public authority(ies), the entity shall be the country's *National Olympic Committee* or its designee.

**National Olympic Committee:** The organization recognized by the International Olympic Committee. The term *National Olympic Committee* shall also include the National Sport Confederation in those countries where the National Sport Confederation assumes typical *National Olympic Committee* responsibilities in the anti-doping area.

**Out-of-Competition:** Any *Doping Control* which is not *In-competition*.

**Person:** A natural person or an organization or other entity.

**Prohibited List:** The List identifying the *Prohibited Substances* and *Prohibited Methods*.

**Prohibited Method:** Any method so described on the *Prohibited List*.

**Prohibited Substance:** Any substance so described on the *Prohibited List*.

**Publicly Disclose or Publicly Report:** To disseminate or distribute information to the general public or *Persons* beyond those *Persons* entitled to earlier notification in accordance with Article 14.

**Sample/Specimen:** Any biological material collected for the purposes of *Doping Control*.

**Signatories:** Those entities signing the *Code* and agreeing to comply with the *Code*, including the International Olympic Committee, International Federations, International Paralympic Committee, *National Olympic Committees*, National Paralympic Committees, *Major Event Organizations*, *National Anti-Doping Organizations*, and WADA.

**Testing:** The parts of the *Doping Control* process involving test distribution planning, *Sample* collection, *Sample* handling, and *Sample* transport to the Laboratory.

**Use:** The application, ingestion, injection or consumption by any means whatsoever of any *Prohibited Substance* or *Prohibited Method*.

**WADA:** The World Anti-Doping Agency.

### 3.2 Defined Terms from the *International Standard for Laboratories*

**Aliquot:** A portion of the *Sample* of biological fluid or tissue (e.g., urine, blood, etc.) obtained from the *Athlete* used in the testing process.

**Certified Reference Material:** Reference Material, accompanied by a certificate, one or more whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

**Confirmation Procedure:** An analytical test procedure whose purpose is to identify the presence of a specific *Prohibited Substance* in a *Sample*. [Comment: A *Confirmation Procedure* may also indicate a quantity of *Prohibited Substance* greater than a threshold value or quantify the amount of a *Prohibited Substance* in a *Sample*.]

**Flexible Accreditation:** Approval for a Laboratory to make restricted modifications in the scope of the accreditation without the involvement of the national accreditation body before the modifications are implemented

**Intermediate Precision,  $s_z$ :** Variation in results observed when one or more factors, such as time, equipment, and operator are varied within a Laboratory with  $i$  denoting the number of factors varied.

**Laboratory Internal Chain of Custody:** Documentation of the sequence of *Persons* in possession of the *Sample* and any portions of the *Sample* taken for Testing. [Comment: *Laboratory Internal Chain of Custody* is generally documented by a written record of the date, location, action taken, and the individual performing an action with a *Sample* or *Aliquot*.]

**Laboratory:** An accredited laboratory applying test methods and processes to provide evidentiary data for the detection and, if applicable, quantification of a Threshold Substance on the *Prohibited List* in urine and other biological *Samples*.

**Laboratory Documentation Packages:** The material produced by the Laboratory to support the finding of an *Adverse Analytical Finding* as set forth in the WADA Technical Document for Laboratory Documentation Packages.

**Minimum Required Performance Limit:** A concentration of a *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method* that a doping Laboratory is expected to reliably detect in the routine daily operation of the Laboratory. See Technical Document Minimum Required Performance Limits for Detection of Prohibited Substances.

**Non-threshold Substance:** A substance listed on the *Prohibited List* for which the documentable detection of any amount is considered an anti-doping rule violation.

**Presumptive Analytical Finding:** The status of a *Sample* test result for which there is an adverse screening test, but a confirmation test has not been performed.

**Reference Collection:** A collection of samples of known origin that may be used in the determination of the identity of an unknown substance. For example, a well characterized sample obtained from a verified administration study in which scientific documentation of the identity of *Metabolite(s)* can be demonstrated.

**Reference Material:** Material or substance one or more of whose properties are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method or for assigning values to materials.

**Repeatability,  $s_r$ :** Variability observed within a laboratory, over a short time, using a single operator, item of equipment, etc.

**Reproducibility,  $s_R$ :** Variability obtained when different laboratories analyze the same *Sample*.

**Revocation:** The permanent withdrawal of a *Laboratory's* WADA accreditation.

**Screening Procedure:** An analytical test procedure whose purpose is to identify those *Samples* which are suspicious with respect to containing a *Prohibited Substance* or *Metabolite* or *Marker* of a *Prohibited Method* and which require additional confirmation testing.

**Split Sample:** Division of a *Sample* taken for testing into two portions at collection, usually designated "A" and "B."

**Suspension:** The temporary withdrawal of a *Laboratory's* WADA accreditation.

**Testing Authority:** The International Olympic Committee, World Anti-Doping Agency, International Federation, National Sport Organization, *National Anti-Doping Organization*, *National Olympic Committee*, *Major Event Organization*, or other authority defined by the Code responsible for *Sample* collection and transport either *In-Competition* or *Out-of-Competition* and/or for management of the test result.

**Threshold Substance:** A substance listed in the *Prohibited List* for which the detection of an amount in excess of a stated threshold is considered an *Adverse Analytical Finding*.

## **PART TWO: LABORATORY ACCREDITATION REQUIREMENTS AND OPERATING STANDARDS**

### **4.0 Requirements for WADA accreditation**

#### **4.1 Initial WADA accreditation**

This section describes the specific requirements for the initial WADA accreditation of the laboratory. All the requirements must be fulfilled in order to obtain an initial WADA accreditation. For some of the requirements, the laboratory has to demonstrate compliance during the probationary period and for other requirements compliance will be checked and controlled based on an accreditation audit (ref. 5.1, 5.2 and 5.3).

##### **4.1.1 ISO/IEC 17025**

The laboratory shall be accredited by a relevant national accreditation body according to ISO/IEC 17025 with primary reference to the interpretations and applications of the ISO/IEC 17025 requirements as they are described in Application of ISO/IEC 17025 to the Analysis of *Doping Control Samples* (Section 5). The ISO/IEC 17025 accreditation must be obtained before the initial WADA accreditation will be given.

##### **4.1.2 Letter of support**

The laboratory shall provide an official letter of support from the relevant national public authority responsible for the national anti-doping program, if any, or a similar letter of support from the *National Olympic Committee* or *National Anti-Doping Organization*. The letter of support shall contain as a minimum:

- Guarantee of sufficient financial support annually for a minimum of 3 years
- Guarantee of sufficient numbers of *Samples* annually for 3 years
- Guarantee of provision of necessary analytical facilities and instrumentation, where applicable

In addition, any explanation of exceptional circumstances shall be given due consideration by WADA. The three year letter of support does not in any way require exclusive support for only one laboratory.

Letters of support from international sport organizations such as International Federations could also be provided in addition to the above mentioned letters.

If the laboratory as an organization is linked to host organizations, (e.g. universities, hospitals...) an official letter of support from the host organizations shall be provided which should include the following information:

- Documentation of the administrative support for the laboratory
- Financial support for the laboratory, if relevant

- Support for the research and development activities
- Guarantee of provision of necessary analytical facilities and instrumentation

#### **4.1.3 Code of Ethics**

The laboratory shall sign and comply with the provision in the Code of Ethics (Annex B) which are relevant for a laboratory in the probationary period.

#### **4.1.4 Proficiency testing program**

During the probationary period the laboratory shall successfully analyze at a minimum four sets of proficiency testing samples containing at a minimum five samples per set.

The final accreditation test shall assess both the scientific competence and the capability of the laboratory to manage multiple samples.

#### **4.1.5 Sharing of knowledge**

The laboratory shall demonstrate during the probationary period its willingness and ability to share knowledge with other WADA Accredited Laboratories. A description of this sharing is provided in the Code of Ethics (Annex B).

#### **4.1.6 Research**

The laboratory shall demonstrate in its budget an allocation to research and development activities in the field of *Doping Control* of at least 7% of the annual budget for the initial 3-year period. The research activities can either be conducted by the laboratory or in cooperation with other WADA-accredited Laboratories or other research organizations.

#### **4.1.7 Initial accreditation of Laboratories holding IOC accreditation**

Laboratories accredited by the IOC in 2003 and which successfully complete the joint 2003 IOC/WADA re-accreditation test and at a minimum conduct an internal audit against Section 5 of the *Internal Standard for Laboratories* will receive WADA accreditation in 2004. The *International Standards for Laboratories* requirements will be fully in effect on January 1<sup>st</sup>, 2004. Laboratories that are downgraded or fail the 2003 IOC/WADA re-accreditation test will have their accreditation suspended or revoked by WADA in accordance with Section 6.4.8. Laboratories which have applied for, but have not received, IOC accreditation will complete their probationary period under the *International Standards for Laboratories*.

## **4.2 Maintaining WADA Accreditation**

This section describes the specific requirements for a WADA re-accreditation of the Laboratory.

#### **4.2.1 ISO/IEC 17025 accreditation**

The Laboratory shall document a valid accreditation from the national accreditation body according to ISO/IEC 17025 with primary reference to the interpretations and applications of the ISO/IEC 17025 requirements as described in the Application of ISO/IEC 17025 to Analysis of *Doping Control Samples* (Section 5).

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#### **4.2.2 Flexible Accreditation**

WADA accredited Laboratories may add or modify scientific methods or add analytes without the need for approval by the body that completed the ISO/IEC 17025 accreditation of that Laboratory. Any analytical method or procedure must be properly selected and validated and included in the scope of the Laboratory at the next ISO audit if the method is used for analysis of *Doping Control Samples*.

#### **4.2.3 Letter of support**

The Laboratory shall provide a renewed official letter of support from the relevant national public authority responsible for the national anti-doping program, if any, or a similar letter of support from the *National Olympic Committee* or *National Anti-Doping Organization* in years in which the Laboratory undergoes an ISO re-accreditation audit. The renewed letter of support shall contain as a minimum:

- Guarantee of sufficient financial support annually for a minimum of 3 years
- Guarantee of sufficient numbers of *Samples* annually
- Guarantee of provision of necessary analytical facilities and instrumentation, where applicable

Any explanation of exceptional circumstances shall be given due consideration by WADA. The letter of support does not in any way require exclusive support for only one Laboratory.

Letters of support from international sport organizations such as International Federations could also be provided in addition to the above mentioned letters.

If the Laboratory as an organization is linked to host organizations (e.g. university, hospital...), an official letter of support from the host organizations shall be renewed for each year in which the Laboratory undergoes a ISO re-accreditation audit and shall include the following information:

- Documentation of the administrative support for the Laboratory
- Financial support for the Laboratory, if relevant
- Guarantee of provision of necessary analytical facilities and instrumentation
- Support for the research activities

#### **4.2.4 Minimum number of testing Samples**

The Laboratory shall periodically provide, at the request of WADA a report documenting all test results reported in a format to be specified by WADA.

In order to maintain proficiency, WADA-accredited Laboratories are required to analyze a minimum of 1500 *Doping Control Samples* per year that are provided by a Testing Authority. If the Laboratory fails to analyze this number of *Samples*, accreditation will be suspended or revoked, dependent on the circumstances.

#### **4.2.5 Proficiency testing program**

The Laboratory are required to successfully participate in the WADA Proficiency Testing program. The program is described in more detail in Annex A.

#### **4.2.6 Reporting**

The Laboratory shall simultaneously report to WADA and the relevant International Federation all *Adverse Analytical Findings* that have been reported to a Testing Authority. All reporting shall be in accord with the confidentiality requirements of the Code.

#### **4.2.7 Code of Ethics**

The Laboratory shall provide documentation of compliance with the provisions of the Code of Ethics (Annex B) relevant for a WADA accredited Laboratory. The Laboratory Director shall send a letter of compliance to WADA every year.

#### **4.2.8 Sharing of knowledge**

The Laboratory shall demonstrate their willingness and ability to share knowledge with other WADA Accredited Laboratories. A description of this sharing is provided in the Code of Ethics (Annex B).

#### **4.2.9 Research**

The Laboratory shall maintain an updated 3-year plan for research and development in the field of *Doping Control*, including an annual budget in this area.

The Laboratory should document the publication of results of the research in relevant scientific papers in the peer-reviewed literature. These documents shall be made available to WADA upon request. The Laboratory may also demonstrate a research program by documenting successful or pending applications for research grants.

### **4.3 Special Requirements for Major Events**

The Laboratory support for the Olympic Games and other major *Events* may be such that the accredited Laboratory facilities are not adequate. This may require relocation of the Laboratory to a new facility, the addition of personnel, or the acquisition of additional equipment. The Laboratory Director of the WADA-accredited Laboratory designated to perform the testing shall be responsible to ensure that the quality management system is maintained.

#### **4.3.1 Satellite facility of an accredited Laboratory**

If the Laboratory is required to move or extend its operation temporarily to a new physical location, the Laboratory must demonstrate a valid ISO/IEC 17025 accreditation with primary compliance with the Application of ISO/IEC 17025 to the Analysis of *Doping Control Samples* for the new facility ("satellite facility").

Any methods or equipment unique to the satellite facility must be validated prior to the satellite facility accreditation audit. Any changes to methods or other procedures in the quality manual must also be validated prior to the audit.

#### 4.3.2 Personnel

The Laboratory shall report to WADA any senior personnel (e.g., certifying scientists, quality system management staff, supervisors, etc.) temporarily working in the Laboratory. The Laboratory Director shall ensure that these personnel are adequately trained in the methods, policies, and procedures of the Laboratory. Particular emphasis should be given to the Code of Ethics and the confidentiality of the results management process. Adequate documentation of training of these temporary employees should be maintained by the Laboratory.

#### 4.3.3 Proficiency testing

WADA may, at its sole discretion, submit proficiency testing samples to the Laboratory for analysis. The samples shall be analyzed by the same methods used in the testing of *Samples* from a Testing Authority. These samples may be part of the ISO/IEC 17025 audit in conjunction with the national accrediting body. Failure(s) to successfully complete the proficiency test will be considered by WADA in deciding whether to accredit the Laboratory. In the event of an unacceptable report, the Laboratory shall document the changes instituted to remedy the failure.

The proficiency testing process should include any additional personnel that are added to the staff for the major *Event*. The samples should be analyzed using the protocols and procedures that will be used for analysis of *Samples* for the *Event*.

#### 4.3.4 Reporting

The Laboratory shall document that the reporting of test results maintains confidentiality.

## 5.0 Application of ISO 17025 to the Analysis of Doping Control Samples

### 5.1 Introduction and Scope

This section of the document is intended as an application as described in Annex B.4 (Guidelines for establishing applications for specific fields) of ISO/IEC 17025 for the field of *Doping Control*. Any aspect of testing or management not specifically discussed in this document shall be governed by ISO/IEC 17025 and, where applicable, by ISO 9001. The application focuses on the specific parts of the processes that are critical with regard to the quality of the laboratory's performance as a *Doping Control Laboratory*. These processes have been determined to be critical to the defined ISO 17025 criteria and are therefore determined to be significant in the evaluation and accreditation process.

This section introduces the specific performance standards for a *Doping Control Laboratory*. The conduct of testing is considered a process within the definitions of ISO 9001. Performance standards are defined according to a process model where the *Doping Control Laboratory* practice is structured into three main categories of processes:



- Analytical and technical processes
- Management processes
- Support processes

Wherever possible, the application will follow the format of the ISO 17025 document. The concepts of the quality management system, continuous improvement, and customer satisfaction included in ISO 9001 have been included.

## 5.2 Analytical and Technical Processes

### 5.2.1 Receipt of Samples

5.2.1.1 Samples may be received by any method authorized by the International Standard for Testing.

5.2.1.2 The transport container shall first be inspected and any irregularities recorded.

5.2.1.3 The name and signature (or other means of identification and recording) of the Person delivering or transferring custody of the shipped Samples, the date, the time of receipt, and the name and signature of the Laboratory representative receiving the Samples, shall be documented as part of the Laboratory Internal Chain of Custody record.

### 5.2.2 Handling of Samples

5.2.2.1 The Laboratory shall have a system to uniquely identify the Samples and associate each Sample with the collection document or other external chain of custody.

5.2.2.2 The Laboratory shall have Laboratory Internal Chain of Custody procedures to maintain control of and accountability for Samples from receipt through final disposition of the Samples. The procedures must incorporate the concepts presented in the WADA Technical Document for Laboratory Internal Chain of Custody (Annex C).

5.2.2.3 The Laboratory shall observe and document conditions that exist at the time of receipt that may impact on the integrity of a Sample report. For example, irregularities noted by the Laboratory should include, but are not limited to:

- Sample tampering is evident.
- Sample is not sealed with tamper-resistant device or seal upon receipt.
- Sample is without a collection form (including Sample identification code) or a blank form is received with the Sample.
- Sample identification is unacceptable. For example, the number on the bottle does not match the Sample identification number on the form.
- Sample volume is extremely low

5.2.2.4 The Laboratory should notify and seek advice from the Testing Authority regarding rejection and testing of Samples for which irregularities are noted.

5.2.2.5 The Laboratory shall retain the A and B Sample(s) for a minimum of three (3) months after the Testing Authority receives a negative report. The Samples shall be retained frozen under appropriate conditions.

Samples with irregularities shall be held frozen for a minimum of three (3) months following the report to the Testing Authority.

5.2.2.6 The Laboratory shall retain the Sample(s) with an Adverse Analytical Finding for a minimum of three (3) months after the Testing Authority receives the final analytical (A or B Sample) report. The Sample shall be stored frozen under appropriate conditions during the long term storage.

5.2.2.7 If the Laboratory has been informed by the Testing Authority that the analysis of a Sample is challenged or disputed, the Sample shall be retained frozen under appropriate conditions and all the records pertaining to the Testing of that Sample shall be stored until completion of any challenges.

5.2.2.8 The Laboratory shall maintain a policy pertaining to retention, release, and disposal of Samples or Aliquots.

5.2.2.9 The Laboratory shall maintain custody information on the transfer of Samples, or portions thereof to another Laboratory.

### **5.2.3 Sampling and Preparation of Aliquots for Testing**

5.2.3.1 The Laboratory shall maintain Laboratory Internal Chain of Custody procedures for control of and accountability for all Aliquots from preparation through disposal. The procedures must incorporate the concepts presented in the WADA Technical Document for Laboratory Internal Chain of Custody.

5.2.3.2 Before the initial opening of a Sample bottle, the device used to ensure integrity of the Sample (e.g., security tape or a bottle sealing system) shall be inspected and the integrity documented.

5.2.3.3 The Aliquot preparation procedure for any Screening Procedure or Confirmation Procedure shall ensure that no risk of contamination of the Sample or Aliquot exists.

### **5.2.4 Testing**

5.2.4.1 Urine integrity testing

5.2.4.1.1 The Laboratory must have a written policy establishing the procedures and criteria for Sample integrity tests.

5.2.4.1.2 The Laboratory should note any unusual condition of the urine – for example: color, odor, or foam. Any unusual conditions should be recorded and included as part of the report to the Testing Authority.

5.2.4.1.3 The Laboratory shall test for the pH and specific gravity as urine integrity parameters on the "A" Sample. Other tests may be performed if requested by the Testing Authority and approved by WADA.

#### 5.2.4.2 Urine screen testing

5.2.4.2.1 The Screening Procedure(s) shall detect the Prohibited Substance(s) or Metabolite(s) of Prohibited Substance(s), or Marker(s) of the Use of a Prohibited Substance or Method for all substances listed in the Out-of-Competition or In-competition Section of the Prohibited List as appropriate for which there is a WADA-accepted screening method. WADA may make specific exceptions to this section.

5.2.4.2.2 The Screening Procedure shall be performed with a WADA-accepted validated method that is appropriate for the substance or method being tested. The criteria for accepting a screening result and allowing the testing of the Sample to proceed must be scientifically valid.

5.2.4.2.3 All screening assays shall include negative and positive controls in addition to the Samples being tested.

5.2.4.2.4 For analytes that must exceed a threshold for reporting as an Adverse Analytical Finding, appropriate controls shall be included in the screening assay. Screening Procedures for Threshold Substances are not required to meet quantitative or uncertainty requirements.

#### 5.2.4.3 Urine confirmation testing

All Confirmation Procedures must be documented and meet applicable uncertainty requirements. The objective of a Confirmation Procedure is to ensure the identification and/or quantification and to exclude any technical deficiency in the Screening Procedure. Since the objective of the confirmation assay is to accumulate additional information regarding an adverse finding, a Confirmation Procedure should have greater selectivity/discrimination than a Screening Procedure.

#### 5.2.4.3.1 "A" Sample Confirmation

5.2.4.3.1.1 Presumptive identification from a Screening Procedure of a *Prohibited Substance*, *Metabolite(s)* of a *Prohibited Substance*, or *Marker(s)* of the *Use of a Prohibited Substance or Method* must be confirmed using a second Aliquot(s) taken from the original "A" Sample.

5.2.4.3.1.2 Mass spectrometry coupled to either gas or liquid chromatography is the method of choice for confirmation of *Prohibited Substances*, *Metabolite(s)* of a *Prohibited Substance*, or *Marker(s)* of the *Use of a Prohibited Substance or Method*. GC/MS or HPLC/MS are acceptable for both Screening Procedures and Confirmation Procedures for a specific analyte.

5.2.4.3.1.3 Immunoassay for confirmation of prohibited proteins, peptides, mimetics, and analogues or *Marker(s)* of their *Use* is permitted. The immunoassay used for confirmation must use a procedure with a different antibody that should recognise a different epitope of the peptide/protein than the assay used for screening.

5.2.4.3.1.4 The Laboratory must have a policy to define those circumstances where the confirmation testing of an "A" Sample may be repeated (e.g., batch quality control failure). Each repeat confirmation must be documented and be completed on a new Aliquot of the "A" Sample.

5.2.4.3.1.5 The Laboratory is not required to confirm every *Prohibited Substance* that is identified by the Screening Procedures. The decision on the prioritization on order of confirmation(s) should be made in cooperation with the Testing Authority and the decision documented. In addition, no Certificate of Analysis or final written Test Report incorporating a Presumptive Analytical Finding shall be issued.

#### 5.2.4.3.2 "B" Sample Confirmation

5.2.4.3.2.1 In those cases where confirmation of a *Prohibited Substance*, *Metabolite(s)* of a *Prohibited Substance*, or *Marker(s)* of the *Use of a Prohibited Substance or Method* is requested in the "B" Sample, the "B" Sample analysis should occur as soon as possible and should be completed within thirty (30) days of notification of an "A" Sample *Adverse Analytical Finding*.

5.2.4.3.2.2 The "B" Sample confirmation must be performed in the same Laboratory as the "A" Sample confirmation. A different

analyst must perform the "B" analytical procedure. The same individual(s) that performed the "A" analysis may perform instrumental set up and performance checks and verify results.

5.2.4.3.2.3 The B *Sample* result must confirm the A *Sample* identification for the *Adverse Analytical Finding* to be valid. The mean value for the B *Sample* finding for Threshold Substances is required to exceed that threshold including consideration of uncertainty.

5.2.4.3.2.4 The *Athlete and/or a representative*, a representative of the entity responsible for *Sample* collection or results management, a representative of the *National Olympic Committee*, *National Sport Federation*, *International Federation*, and a translator shall be authorized to attend the "B" confirmation.

In the absence of all of the above persons, the Testing Authority or the Laboratory shall appoint a surrogate (independent witness) to verify that the "B" *Sample* container shows no signs of tampering and that the identifying numbers match that on the collection documentation.

The Laboratory Director may limit the number of individuals in Controlled Zones of the Laboratory based on safety or security considerations.

The Laboratory Director may remove, or have removed by proper authority, any *Athlete* or representative that is interfering in the testing process. Any behavior resulting in removal should be reported to the Testing Authority and may be considered anti-doping rule violation in accordance with Article 2.5 of the Code, "Tampering, or Attempting to tamper, with any part of *Doping Control*".

5.2.4.3.2.5 Aliquots taken for analysis must be taken from the original "B" *Sample*.

5.2.4.3.2.6 The Laboratory must have a policy to define those circumstances when confirmation testing of the "B" *Sample* may be repeated. Each repeat confirmation should be performed on a new Aliquot of the "B" *Sample*.

5.2.4.3.2.7 If the "B" *Sample* confirmation does not provide analytical findings that confirm the "A" *Sample* result, the *Sample* shall be considered negative and the Testing Authority notified of the new analytical finding.

#### 5.2.4.4 Alternative biological matrices screening and confirmatory testing

5.2.4.4.1 Unless otherwise defined, this application applies only to the analysis of urine *Samples*. Blood, plasma, and serum are acceptable matrices for testing in certain circumstances. Specific requirements for the testing of these matrices are not included in the scope of this document and will be promulgated separately.

5.2.4.4.2 Any testing results of hair, nails, oral fluid or other biological material shall not be used to counter *Adverse Analytical Findings* from urine.

### 5.2.5 Results Management

#### 5.2.5.1 Review of results

5.2.5.1.1 A minimum of two certifying scientists must independently review all *Adverse Analytical Findings* before a report is issued. The review process shall be documented.

5.2.5.1.2 At a minimum, the review shall include:

- Laboratory Internal Chain of Custody documentation
- Urine integrity data
- Validity of the analytical screening and confirmation data and calculations
- Quality control data
- Completeness of documentation supporting the reported analytical findings

5.2.5.1.3 When an *Adverse Analytical Finding* is rejected, the reason(s) must be documented.

#### 5.2.6 Documentation and Reporting

5.2.6.1 The Laboratory must have documented procedures to ensure that it maintains a coordinated record related to each *Sample* analyzed. In the case of an *Adverse Analytical Finding*, the record must include the data necessary to support the conclusions reported (as set forth in the Technical Document; Laboratory Documentation Packages). In general, the record should be such that in the absence of the analyst, another competent analyst could evaluate what tests had been performed and interpret the data.

5.2.6.2 Each step of testing shall be traceable to the staff member who performed that step.

5.2.6.3 Significant variance from the written procedure shall be documented as part of the record (e.g., memorandum for the record).

5.2.6.4 Where instrumental analyses are conducted, the operating parameters for each run shall be recorded.

5.2.6.5 Reporting of "A" *Sample* results should occur within ten (10) working days of receipt of the *Sample*. The reporting time required for specific competitions may be substantially less than ten days. The reporting time may be modified by agreement between the Laboratory and the Testing Authority.

5.2.6.6 The Laboratory Certificate of Analysis or Test Report shall include, in addition to the items stipulated in ISO 17025, the following:

- *Sample* identification number
- Laboratory Identification number (if any)
- Status of test (*Out of competition/In-competition*)
- Name of competition and/or sport
- Date of receipt of *Sample*
- Date of report
- Type of sample (urine, blood, etc.)
- Test results
- Signature of certifying individual
- Other information as specified by the Testing Authority.

5.2.6.7 The Laboratory is not required to measure or report a concentration for *Prohibited Substances* for a non-threshold analyte. The Laboratory should report the actual *Prohibited Substance(s)*, *Metabolite(s)* of the *Prohibited Substance(s)* or *Method(s)*, or *Marker(s)* detected in the *Sample*.

5.2.6.8 For Threshold Substances, the Laboratory report should establish that the *Prohibited Substance* or its *Metabolite(s)* or *Marker(s)* of a *Prohibited Method* is present at a concentration greater than the threshold concentration taking into consideration the uncertainty in concluding that the concentration in the *Sample* exceeds the threshold. The estimate of uncertainty should not be included on the Certificate of Analysis or Test Report but must be included in Laboratory Documentation Packages.

5.2.6.9 The Laboratory shall have a policy regarding the provision of opinions and interpretation of data. An opinion or interpretation may be included in the Certificate of Analysis or Test Report provided that the opinion or interpretation is clearly identified as such. The basis upon which the opinion has been made shall be documented.

Note: An opinion or interpretation may include, but not be limited to, recommendations on how to use results, information related to the pharmacology, metabolism and pharmacokinetics of a substance, and whether an observed result is consistent with a set of reported conditions.

5.2.6.10 In addition to reporting to the Testing Authority, the Laboratory shall simultaneously report any *Adverse Analytical Findings* to WADA and the responsible International Federation. In the case where the sport or Event is not associated with an International Federation (e.g., college sports) or the *Athletes* are not members of an International Federation, the Laboratory is required to report *Adverse Analytical Findings* only to WADA. All reporting shall be in accord with the confidentiality requirements of the *Code*.

5.2.6.11 The Laboratory shall report quarterly to WADA, in a format specified by WADA, a summary of the results of all tests performed. No information that could link an *Athlete* with an individual result will be included. The report will include a summary of any *Samples* rejected for testing and the reason for the rejection.

When the clearinghouse is in place, the Laboratory shall simultaneously report to WADA all information reported to the Testing Authority, according to the requirements listed in Section 5.2.6.6, in lieu of the paragraph above. The information will be used to generate summary reports.

5.2.6.12 Laboratory Documentation Packages shall contain material specified in the WADA Technical Document on Laboratory Documentation Packages.

5.2.6.13 *Athlete* confidentiality is a key concern for all Laboratories engaged in *Doping Control* cases. Confidentiality requires extra safeguards given the sensitive nature of these tests.

5.2.6.13.1 Testing Authority requests for information must be made in writing to the Laboratories.

5.2.6.13.2 *Adverse Analytical Findings* shall not be provided by telephone.

5.2.6.13.3 Information sent by a facsimile is acceptable if the security of the receiving facsimile machine has been verified and procedures are in place to ensure that the facsimile has been transmitted to the correct facsimile number.

5.2.6.13.4 Unencrypted email is not authorized for any reporting or discussion of *Adverse Analytical Findings* if the *Athlete* can be identified or if any information regarding the identity of the *Athlete* is included. The Laboratory shall also provide any information requested by WADA in conjunction with the Monitoring Program, as set forth in Article 4.5 of the *Code*.



### **5.3 Quality Management Processes**

#### **5.3.1 Organization**

5.3.1.1 Within the framework of ISO/IEC 17025, the Laboratory shall be considered a testing laboratory (and not a calibration laboratory).

5.3.1.2 The Laboratory (Scientific) Director shall have the responsibilities of the Chief Executive, unless otherwise noted.

#### **5.3.2 Quality Policy and Objectives**

5.3.2.1 The Quality Policy and implementation shall meet the requirements of ISO/IEC 17025 Section 4.2 Quality Management System and shall include a quality manual that describes the quality system.

5.3.2.2 A single staff member should be appointed as the Quality Manager and should have responsibility and authority to implement and ensure compliance with the quality system.

#### **5.3.3 Document Control**

The control of documents that make up the Quality Management System shall meet the requirements of ISO/IEC 17025 Section 4.3 Document Control

5.3.3.1 The Laboratory Director (or designee) shall approve the Quality Manual and all other documents used by staff members in completing testing.

5.3.3.2 The Quality Management System shall ensure that the contents of WADA Technical Documents are incorporated into the appropriate manuals by the effective date and that training is provided and documented. If this is not possible, WADA should be contacted with a written request for an extension.

#### **5.3.4 Review of requests, tenders, and contracts**

Review of legal documents or agreements related to testing must meet the requirements of ISO/IEC 17025 Section 4.4.

The Laboratory shall ensure that the Testing Authority is informed concerning the tests that can be performed on Samples submitted for analysis.

#### **5.3.5 Subcontracting of tests**

A WADA-accredited Laboratory must perform all work with its own personnel and equipment within its accredited facility. In the case of specific technologies that may not be available in the Laboratory (e.g., GC/IRMS, Isoelectric focusing [EPO/NESP]), a Sample may be transferred to another WADA-accredited Laboratory in which the technology is within the scope of analysis.

In exceptional circumstances, WADA may elect to grant specific authorization for subcontracting part of the tasks. In such cases, assurance of maintaining the level of quality and the appropriate chain of custody throughout the entire process is the responsibility of the Laboratory Director of the WADA-accredited Laboratory.

### 5.3.6 Purchasing of services and supplies

#### 5.3.6.1 Chemicals and reagents

Chemicals and reagents must be suitable for the purpose and be of established purity. Reference purity documentation must be obtained when available and retained in the quality system documents.

In the case of rare or difficult to obtain reagents, Reference Materials, or Reference Collections, particularly for use in qualitative methods, the expiration date of the solution can be extended if adequate documentation exists that no significant deterioration has occurred.

5.3.6.2 Waste disposal shall be in accord with national laws and other relevant regulations. This includes biohazard materials, chemicals, controlled substances, and radioisotopes, if used.

5.3.6.3 Environmental health and safety policies should be in place to protect the staff, the public, and the environment.

### 5.3.7 Service to the client

5.3.7.1 Service to clients shall be handled in accord with ISO/IEC 17025 Section 4.7.

#### 5.3.7.2 Ensuring responsiveness to WADA

The Laboratory Director or his designee must:

- Ensure adequate communication.
- Report to WADA any unusual circumstances or information with regard to testing programs, patterns of irregularities in Specimens, or potential Use of new substances.
- Provide complete and timely explanatory information to WADA as appropriate and as requested to provide quality accreditation.

#### 5.3.7.3 Ensuring Testing Authority focus

5.3.7.3.1 The Laboratory Director shall be familiar with the Testing Authority rules and the Prohibited List.

5.3.7.3.2 The Laboratory Director should interact with the Testing Authority with respect to specific timing, report information, or other support needs. These interactions should include, but are not limited to, the following:

- Communicate with the Testing Authority concerning any significant question of testing needs or any unusual circumstance in the testing process (including delays in reporting).
- Act without bias regarding the national affiliation of the Testing Authority.
- Provide complete and timely explanations to the Testing Authority when requested or when there is a potential for misunderstanding the Test Report or Certificate of Analysis.
- Provide evidence and/or expert testimony on any test result or report produced by the Laboratory as required in administrative, arbitration, or legal proceedings.
- Respond to any comment or complaint submitted by a Testing Authority or Anti-Doping Organization concerning the Laboratory and its operation.

5.3.7.3.3 The Laboratory shall monitor Testing Authority satisfaction. There should be documentation that the Testing Authority concerns have been incorporated into the Laboratory Quality Management System, where appropriate.

5.3.7.3.4 The Laboratory shall develop a system, as required by ISO 17025, for monitoring key indicators of Laboratory service.

#### **5.3.8 Complaints**

Complaints shall be handled in accord with ISO/IEC 17025 Section 4.8.

#### **5.3.9 Control of nonconforming testing work**

5.3.9.1 The Laboratory shall have policies and procedures that shall be implemented when any aspect of its testing or a result from its testing does not comply to set procedures.

5.3.9.2 Documentation of any non-compliance or deviation from procedure or protocol involving a *Sample* testing shall be kept as part of the permanent record of that *Sample*.

#### **5.3.10 Corrective action**

Corrective action shall be taken in accord with ISO/IEC 17025 Section 4.10.

#### **5.3.11 Preventive action**

Preventive action shall be taken in accord with ISO/IEC 17025 Section 4.11.

#### **5.3.12 Control of records**

##### **5.3.12.1 Technical Records**

5.3.12.1.1 Analytical records on negative *Samples*, including Laboratory Internal Chain of Custody documentation and medical information (T/E ratio, steroid profiles, and blood parameters), must be

retained in secure storage for at least two (2) years. Relevant records on *Samples* with irregularities or rejected *Samples* must be retained in secure storage for at least two (2) years.

5.3.12.1.2 All analytical records on *Specimens* with an *Adverse Analytical Finding* must be retained in secure storage for at least five (5) years, unless otherwise specified by the Testing Authority or by contract.

5.3.12.1.3 The raw data supporting all analytical results must be retained in secure storage for five (5) years.

#### **5.3.13 Internal Audits**

5.3.13.1 Internal audits shall be completed in accordance with the requirements of ISO/IEC 17025 Section 4.13.

5.3.13.2 Internal Audit responsibilities may be shared amongst personnel provided that any *Person* does not audit his/her own area.

#### **5.3.14 Management Reviews**

5.3.14.1 Management reviews will be conducted to meet the requirements of ISO/IEC 17025 Section 4.14.

5.3.14.2 WADA will publish, from time to time, specific technical recommendations in a Technical Document. Implementation of the technical recommendations described in the Technical Documents is mandatory and should occur by the effective date.

Technical Documents supersede any previous publication on a similar topic, or if applicable, this document. The document in effect will be that Technical Document whose effective date most recently precedes that of *Sample* receipt date. The current version of the Technical Document will be available on WADA's website.

### **5.4 Support processes**

#### **5.4.1 General**

General support shall be provided in accord with ISO/IEC 17025.

#### **5.4.2 Personnel**

5.4.2.1 Every person employed by, or under contract to, the Laboratory must have a personnel file accessible for auditors. The file must contain copies of the résumé, or qualification form, a description of the job, and documentation of initial and ongoing training. The Laboratory must maintain appropriate confidentiality of personal information.

- 5.4.2.2 All personnel should have a thorough knowledge of their responsibilities including the security of the Laboratory, confidentiality of results, Laboratory Internal Chain of Custody, protocols, and the standard operating procedures for any method that they perform.
- 5.4.2.3 The Laboratory Director is responsible for ensuring that Laboratory personnel are adequately trained and have experience necessary to perform their duties. The certification should be documented in the individual's personnel file.
- 5.4.2.4 The Doping Control Laboratory must have a qualified person as the Laboratory Director to assume professional, organizational, educational, and administrative responsibility. The Laboratory Director qualifications are:
- Ph.D. or equivalent in one of the natural sciences or Training comparable to a Ph.D. in one of the natural sciences such as a medical or scientific degree with appropriate experience or training.
  - Experience with the analysis of biological material for substances used in doping.
  - Appropriate training or experience in forensic applications of Doping Control.
- 5.4.2.5 The Doping Control Laboratory must have qualified personnel to serve as Certifying Scientist(s) to review all pertinent data, quality control results, and to attest to the validity of the Laboratory's test reports. The qualifications are:
- Bachelors Degree in Medical Technology, Chemistry, Biology, or related natural science or equivalent. Documented experience of 8 years or more in a Doping Control Laboratory is equivalent to a Bachelor's degree for this position.
  - Experience in the analysis of doping materials in biological fluids.
  - Experience in the use of relevant analytical techniques such as chromatography, immunoassay, and Gas Chromatography/Mass Spectrometry.
- 5.4.2.6 Supervisory personnel should have a thorough understanding of the Quality Control procedures; the review, interpretation, and reporting of test results; maintenance of Laboratory Internal Chain of Custody; and proper remedial action to be taken in response to analytical problems. The qualifications for supervisor are:
- Bachelors Degree in Medical Technology, Chemistry, Biology, or related natural science or equivalent. Documented experience of 5 years or more in a Doping Control Laboratory is equivalent to a Bachelor's degree for this position.

- Experience in relevant analytical testing including the analysis of *Prohibited Substances* in biological material.
- Experience in the use of analytical techniques such as chromatography, immunoassay, and Gas Chromatography/Mass Spectrometry.
- Ability to ensure compliance with quality management systems and quality assurance processes.

#### **5.4.3 Accommodation and environmental conditions**

##### **5.4.3.1 Environmental Control**

###### **5.4.3.1.1 Maintain appropriate electrical services**

5.4.3.1.1.1 The Laboratory shall ensure that adequate electrical service is available so that there is no interruption or compromise of stored data.

5.4.3.1.1.2 All computers, peripherals, and communication devices should be supported in such a way that service is not likely to be interrupted.

5.4.3.1.1.3 The Laboratory shall have policies in place to ensure the integrity of refrigerated and/or frozen stored samples in the event of an electrical failure.

5.4.3.1.2 The Laboratory shall have a written safety policy, and compliance with Laboratory safety policies shall be enforced.

5.4.3.1.3 The storage and handling of controlled substances must comply with applicable national legislation.

##### **5.4.3.2 Security of the facility**

5.4.3.2.1 The Laboratory shall have a policy for the security of its facilities, which may include a threat and risk assessment.

5.4.3.2.2 Three levels of access should be considered in the quality manual or threat assessment plan:

- Reception zone. An initial point of control beyond which unauthorized individuals must be escorted.
- Common operational zones.
- Controlled zones. Access to these areas should be monitored and records maintained of access by visitors.

5.4.3.2.3 The Laboratory shall restrict access to Controlled Zones to only authorized persons. A staff member should be assigned as the

security officer who has overall knowledge and control of the security system.

5.4.3.2.4 Unauthorized persons must be escorted within Controlled Zones. A temporary authorization may be issued to individuals requiring access to the Controlled Zones such as auditing teams and individuals performing service or repair.

5.4.3.2.5 It is advisable to have a separate Controlled Zone for *Sample* receipt and Aliquot preparation.

#### 5.4.4 Test Methods and Method Validation

##### 5.4.4.1 Selection of Methods

Standard methods are generally not available for *Doping Control* analyses. The Laboratory shall develop, validate, and document in-house methods for compounds present on the *Prohibited List* and for related substances. The methods shall be selected and validated so they are fit for the purpose.

##### 5.4.4.1.1 Non-threshold Substances

Laboratories are not required to measure or report a concentration for Non-threshold Substances.

The Laboratory must develop as part of the method validation process acceptable standards for identification of *Prohibited Substances*. (See the Technical Document on Identification Criteria for Qualitative Assays)

The Laboratory must demonstrate the ability to achieve the Minimum Required Performance Limits using a representative substance or substances if the appropriate standards are available. In case a Reference Collection is used for identification, an estimate of the limit of detection for the method must be provided by assessing a representative substance.

##### 5.4.4.1.2 Threshold Substances

The Laboratory must develop methods with an acceptable uncertainty near the threshold concentration. The method must be capable of documenting both the relative concentration and the identity of the *Prohibited Substance* or *Metabolite(s)* or *Marker(s)*.

Confirmation methods for Threshold Substances must be performed on three Aliquots from the "A" bottle and three Aliquots from the "B" bottle, if the "B" sample confirmation is performed. If insufficient Sample volume exists to analyze three Aliquots, the maximum number of Aliquots that can be prepared should be analyzed. *Adverse Analytical Finding* decisions shall be based on the mean of the measured

concentrations and include consideration of uncertainty with the coverage factor,  $k$ , reflecting the number of Aliquots analyzed and a level of confidence of 95%. Reports and documentation, where necessary, shall report the mean concentration.

#### 5.4.4.1.3 Minimum Required Performance Limit

For both Non-threshold and Threshold Substances, the Laboratory will be required to meet a Minimum Required Performance Limit for detection, identification, and demonstration that a substance exceeds the threshold (if required).

#### 5.4.4.2 Validation of Methods

##### 5.4.4.2.1 Confirmation methods for Non-threshold Substances must be validated. Examples of factors relevant to determining if the method is fit for the purpose are:

- Specificity. The ability of the assay to detect only the substance of interest must be determined and documented. The assay must be able to discriminate between compounds of closely related structures.
- Identification capability. Since the results for Non-threshold substances are not quantitative, the Laboratory should establish criteria for ensuring that identification of a substance representative of the class of Prohibited Substances can be repeatedly identified and detected as present in the sample at a concentration near the MRPL.
- Robustness. The method must be determined to produce the same results with respect to minor variations in analytical conditions. Those conditions that are critical to reproducible results must be controlled.
- Carryover. The conditions required to eliminate carryover of the substance of interest from sample to sample during processing or instrumental analysis must be determined and implemented.
- Matrix interferences. The method should avoid interference in the detection of Prohibited Substances or their Metabolites or Markers by components of the sample matrix.
- Standards. Reference standards should be used for identification, if available. If there is no reference standard



available, the use of data or sample from a validated Reference Collection is acceptable.

5.4.4.2.2 Confirmation methods for Threshold Substances must be validated. Examples of factors relevant to determining if the method is fit for the purpose are:

- Specificity. The ability of the assay to detect only the substance of interest must be determined and documented. The assay must be able to discriminate between compounds of closely related structures.
- Intermediate Precision. The method must allow for the reliable repetition of the results at different times and with different operators performing the assay. Intermediate Precision at the threshold must be documented.
- Robustness. The method must be determined to produce the same results with respect to minor variations in analytical conditions. Those conditions that are critical to reproducible results must be controlled.
- Carryover. The conditions required to eliminate carryover of the substance of interest from sample to sample during processing or instrumental analysis must be determined and implemented.
- Matrix interferences. The method must limit interference in the measurement of the amount of *Prohibited Substances* or their *Metabolites* or *Markers* by components of the sample matrix.
- Standards. Reference standards should be used for quantification, if available. If there is no reference standard available, the use of data or sample from a validated Reference Collection is acceptable.
- Minimum Required Performance Limits (MRPL). The Laboratory must demonstrate that it can detect representative compounds of each prohibited class at defined MRPLs. The Laboratory should also determine the limit of detection and limit of quantification if the MRPL is close to these limits.
- Linearity must be documented at 50% to 200% of the threshold value, unless otherwise stipulated in a Technical Document.

#### 5.4.4.3 Estimate of Uncertainty of Method

In most cases an identification of a *Prohibited Substance*, its *Metabolite(s)* or *Marker(s)*, is sufficient to report an *Adverse Analytical Finding*. Thus, quantitative uncertainty as defined in ISO/IEC 17025 does not apply. In the identification of a compound by GC/MS or HPLC/MS, there are qualitative measures that substantially decrease the uncertainty of identification.

In the case of a *Threshold Substance*, uncertainty in both the identification and the finding that the substance is present in an amount greater than the threshold concentration must be addressed.

##### 5.4.4.3.1 Uncertainty in identification

The appropriate analytical characteristics must be documented for a particular assay. The Laboratory must establish criteria for identification of a compound at least as strict as those stated in any relevant Technical Document.

##### 5.4.4.3.2 Uncertainty in establishing that a substance exceeds a threshold.

The purpose of threshold reporting in *Doping Control* is to establish that the *Prohibited Substance* or its *Metabolite(s)* or *Marker(s)* are present at a concentration greater than the threshold value. The method, including selection of standards and controls, and report of uncertainty should be designed to fit the purpose.

##### 5.4.4.3.2.1 Uncertainty of quantitative results, particularly at the threshold value, should be addressed during the validation of the assay through measurement of Repeatability, Intermediate Precision and bias, where possible.

##### 5.4.4.3.2.2 The expression of uncertainty should use the expanded uncertainty using a coverage factor, $k$ , to reflect a level of confidence of 95 %. The expression of uncertainty may also take the form of a one-sided t-test at a level of confidence of 95 %.

##### 5.4.4.3.2.3 Uncertainty may be further addressed in Technical Documents in order to reflect the purpose of analysis for the specific substances.

#### 5.4.4.4 Control of Data

##### 5.4.4.4.1 Data and Computer Security

##### 5.4.4.4.1.1 Access to computer terminals, computers, or other operating equipment shall be controlled by physical access and by multiple levels of access controlled by

passwords or other means of employee recognition and identification. These include, but are not limited to account privileges, user identification codes, disk access, and file access control.

5.4.4.4.1.2 The operating software and all files shall be backed up on a regular basis and a current copy kept off site at a secure location.

5.4.4.4.1.3 The software shall prevent the changing of results unless there is a system to document the person doing the editing and that editing can be limited to users with proper level of access.

5.4.4.4.1.4 All data entry, recording of reporting processes and all changes to reported data shall be recorded with an audit trail. This shall include the date and time, the information that was changed, and the individual performing the task.

## **5.4.5 Equipment**

5.4.5.1 A List of available equipment is to be established and maintained.

5.4.5.2 As part of a quality system, the Laboratories shall operate a program for the maintenance and calibration of equipment according to ISO 17025 Section 5.5.

5.4.5.3 General service equipment that is not used for making measurements should be maintained by visual examination, safety checks, and cleaning as necessary. Calibrations are only required where the setting can significantly change the test result. A maintenance schedule shall be established for items such as fume hoods, centrifuges, evaporators, etc, which are used in the test method.

5.4.5.4 Equipment or volumetric devices used in measuring shall have periodic performance checks along with servicing, cleaning, and repair.

5.4.5.5 Qualified subcontracted vendors may be used to service, maintain, and repair measuring equipment.

5.4.5.6 All maintenance, service, and repair of equipment must be documented.

## 5.4.6 Measurement Traceability

### 5.4.6.1 Reference Standards

Few of the available reference drug and drug *Metabolite(s)* are traceable to national or international standards. When available, reference drug or drug *Metabolite(s)* traceable to a national standard or certified by a body of recognized status, such as USP, BP, Ph.Eur. or WHO, should be used. When available, a certificate of analysis or authenticity shall be obtained.

When a reference standard is not certified, the Laboratory shall verify its identity and purity by comparison with published data or by chemical characterization.

### 5.4.6.2 Reference Collections

A collection of samples or isolates may be obtained from a biological matrix following an authentic and verifiable administration of a *Prohibited Substance* or *Method*, providing that the analytical data are sufficient to justify the identity of the relevant chromatographic peak or isolate as a *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method*.

## 5.4.7 Assuring the quality of test results

5.4.7.1 The Laboratory must participate in the WADA Proficiency Testing Program.

5.4.7.2 The Laboratory shall have in place a quality assurance system, including the submission of blind quality control samples, that challenges the entire scope of the testing process (i.e. sample receipt and accessioning through result reporting).

5.4.7.3 Analytical performance should be monitored by operating quality control schemes appropriate to the type and frequency of testing performed by the Laboratory. The range of quality control activities includes:

- Positive and negative controls analyzed in the same analytical run as the Presumptive *Adverse Analytical Finding Sample*.
- The use of deuterated or other internal standards or standard addition.
- Comparison of mass spectra or ion ratios from selected ion monitoring (SIM) to a Reference Material or Reference Collection sample analyzed in the same analytical run
- Confirmation of the "A" and "B" Split Samples.

- Quality control charts using appropriate control limits (e.g.,  $\pm 20\%$  of the target value) depending on the analytical method employed.
- The quality control procedures should be documented in the Laboratory.

## 6.0 Process of WADA Accreditation

This section describes the technical and financial requirements the laboratory must fulfill in the process of being accredited by WADA. The description of the steps in the accreditation process is linked to the defined requirement presented in Section 4.

### 6.1 Applying for a WADA Laboratory Accreditation

#### 6.1.1 Submit Application Form

The laboratory must fill in the necessary information in the Application Form as provided by WADA and deliver this to WADA with the required documentation and applicable fee. The Application shall be signed by the Laboratory Director and, if relevant, by the Director of the host organization.

#### 6.1.2 Description of Laboratory

As preparations for an initial visit by WADA, the laboratory shall complete a questionnaire provided by WADA and submit it to WADA no later than four weeks after the receipt of the questionnaire. The following information shall be submitted through the questionnaire:

- List of staff and their qualifications
- Description of physical facilities, including a description of the security considerations for Samples and records
- List of proposed and actual instrumental resources and equipment
- List of available Reference Materials or standards, or plans to acquire Reference Materials or standards, including properly validated biological Sample Reference Collections
- Financial or business plan for the laboratory

WADA may require an update of this documentation during the process of accreditation.

#### 6.1.3 Provide a letter of support

According to 4.1.2 the laboratory shall provide necessary letters of support containing the required information from the relevant national public authorities, or *National Olympic Committee*, or *National Anti-Doping Organization*.

#### 6.1.4 Conduct Initial visit

If necessary, WADA shall conduct an initial visit (2-3 days) to the laboratory at the laboratory's expense. The purpose of this visit is to clarify issues with regard to the accreditation process and the defined requirements in the *International Standard* for

Laboratories and to obtain information about different aspects of the laboratory relevant for the accreditation.

#### **6.1.5 Issue final report and recommendation**

Within eight (8) weeks after the initial visit or the receipt of the questionnaire, WADA will complete and submit a report to the laboratory. In the report WADA will make the necessary recommendations concerning giving the laboratory status as a WADA Probationary laboratory or if this is not the case, identifying needed improvements in order to be a WADA Probationary laboratory.

#### **6.2 Preparing for WADA Laboratory Accreditation**

A probationary period shall be defined for a WADA Probationary Laboratory. The period will range from 12 to 24 months depending on the status of the laboratory with regard to the defined requirements (refer to Section 4.1). The main purpose of this period is that the laboratory shall prepare for initial accreditation. During this period, WADA will provide appropriate feedback to assist the laboratory in improving the quality of its testing process. In this period the laboratory shall:

##### **6.2.1 Obtain ISO 17025 accreditation**

The laboratory shall prepare and establish the required documentation and system according to the requirements in Application of ISO 17025 to Analysis of *Doping Control Sample* (Section 5) and the ISO 17025. Based on this, the laboratory shall initiate and prepare for the accreditation process by consulting with a relevant national accreditation body. An audit team consisting of representatives from a national accreditation body, including independent technical assessors recommended by WADA will audit the laboratory. Copies of the Audit Report shall be sent to WADA. The laboratory has to correct any identified non-conformities within defined time-frames and document this accordingly. Copies of the documentation of the correction of the non-conformities should be sent to WADA.

##### **6.2.2 Participate in the WADA Proficiency Testing Program**

The laboratory must complete a minimum of one year of successful participation in the WADA Proficiency Testing program prior to achieving initial accreditation. (See Annex A for description of the Proficiency Testing program.)

As a final proficiency test, the laboratory shall analyze 20-50 urine *Samples* in the presence of a WADA representative. Costs associated with the WADA on-site visit shall be at the laboratory's expense. The laboratory shall successfully identify and/or document a concentration in excess of the threshold of all of the *Prohibited Substances, Metabolite(s) of Prohibited Substances, or Marker(s) of Prohibited Substances* or Methods within five (5) days of the laboratory opening the *Samples*. The laboratory shall provide a Certificate of Analysis for each of the *Samples* in the proficiency test. For negative *Samples*, WADA may request all or a portion of the negative screening data. For each of the *Samples* for which there is an *Adverse Analytical Finding*, the laboratory shall provide a Laboratory Documentation Package. This data shall be submitted within two (2) weeks of submission of the initial report.

### **6.2.3 Implement Code of Ethics**

The laboratory shall communicate the Code of Ethics (Annex B) to all employees and ensure understanding of and commitment to the different aspects of the Code of Ethics.

### **6.2.4 Plan and implement research activities**

The laboratory shall develop a plan for its research and development activities in the field of *Doping Control* within a 3 year period including a budget. At least two research and development activities shall be initiated and implemented within the probationary period.

### **6.2.5 Plan and implement sharing of knowledge**

The laboratory shall prepare and convey information and knowledge on at least two specific issues to the other WADA accredited Laboratories within the probationary period.

## **6.3 Obtaining WADA Accreditation**

### **6.3.1 Participate in a WADA accreditation audit**

In the last phase of the probationary period WADA will prepare in cooperation with the laboratory a final WADA accreditation audit. Representatives of WADA will audit compliance of the defined requirements in the Application of ISO 17025 to Analysis of *Doping Control Samples* (Section 5) and the practice and documentation of the laboratory. If WADA has participated in the initial ISO audit, the final WADA audit may be a document audit. Otherwise, the audit can be conducted together with the national accreditation body or separately if more practical. Should an on-site audit take place by WADA, the associated cost shall be at the laboratory's expense. Based on the audit, WADA will issue an Audit Report and submit this to the laboratory. If needed, the laboratory will have to correct identified non-compliances within defined time-frames and report these to WADA.

### **6.3.2 WADA report and recommendation**

Based on the relevant documentation from the laboratory, any WADA technical advisor feedback, and the relevant accreditation body (Audit Report), WADA will make a final report including a recommendation concerning the accreditation of the laboratory. The report and recommendation will be submitted to the WADA Executive Committee for approval. In case that the recommendation is that the laboratory should not be accredited, the laboratory will have a maximum of six (6) months to correct and improve specific parts of their operation, at which time a further report will be made by WADA.

### **6.3.3 Issue and publication of Accreditation certificate**

A certificate signed by a duly authorized representative of WADA shall be issued in recognition of an accreditation. Such certificate shall specify the name of the Laboratory and the period for which the certificate is valid. Certificates may be

issued after the effective date, with retroactive effect. A list of accredited Laboratories will be published annually by WADA.

## **6.4 Maintaining WADA Accreditation**

### **6.4.1 Provide a new letter of support**

Letter(s) of Support from a national public authority or *National Olympic Committee* or *National Anti-Doping Organization* responsible for a national *Doping Control* program or an International Federation responsible for an international *Doping Control* program shall be required in years in which there is an ISO 17025 re-accreditation audit.

A letter of support from the host organization renewing its commitment to the Laboratory shall also be required in conjunction with each ISO 17025 re-accreditation audit.

### **6.4.2 Document annual number of tests**

The Laboratory shall periodically report the results of all tests performed to WADA in a specified format. WADA will monitor *Sample* test volume performed by the Laboratory. If the number of *Samples* falls below 1500 per year, WADA Laboratory accreditation will be suspended or revoked in accordance with Section 6.4.8.

### **6.4.3 Flexible Accreditation**

WADA accredited Laboratories may add or modify scientific methods or add analytes to its scope of work without the need for approval by the body that completed the ISO/IEC 17025 accreditation of that Laboratory. Any analytical method or procedure must be properly selected and validated and included in the scope of the Laboratory at the next ISO audit if use is continued.

### **6.4.4 Document Compliance with the WADA Laboratory Code of Ethics**

The Laboratory Director must send a letter of compliance to WADA every year. The Laboratory may be asked to provide documentation of compliance with the provisions of the Code of Ethics (Annex B).

### **6.4.5 Document implemented research activities**

The Laboratory must supply an annual progress report to WADA documenting research and development results in the field of *Doping Control* and dissemination of the results. The Laboratory should also relate research and development plans for the next year.

### **6.4.6 Document implemented sharing of knowledge**

The Laboratory must supply an annual report sharing of knowledge with all other WADA-accredited Laboratories.



#### **6.4.7 Participate in WADA/ISO periodical audits and the re-accreditation audit**

WADA reserves the right to inspect and audit the Laboratory at any time. The notice of the audit/inspection will be made in writing to the Laboratory Director. In exceptional circumstances, the audit/inspection may be unannounced.

##### **6.4.7.1 WADA/ISO Re-accreditation audit**

The Laboratory must receive ISO/IEC 17025 accreditation including compliance with the Application of ISO 17025 for Analysis of Doping Control Samples (Section 5 of this document). The audit team may include a WADA Consultant to augment the auditing team selected by the national accrediting body for the re-accreditation audit.

Copies of the audit summary report as well as the Laboratory responses must be sent to WADA. The Laboratory shall also provide a copy of the ISO 17025 certificate obtained from the national certifying body.

##### **6.4.7.2 ISO Periodical audit**

In years when a periodical ISO/IEC 17025 audit is required, the Laboratory shall provide WADA with a copy of any external audits and evidence of corrective actions for any non-compliance.

#### **6.4.8 WADA report and recommendation**

WADA will annually review Laboratory compliance with the requirements listed in Sections 4 and 5. With the exception of re-accreditation and other required on-site audits, the annual review will consist of a documentation audit. WADA may require documentation from the Laboratory. Failure of the Laboratory to provide information requested in evaluating performance by the specified date shall be considered a refusal to cooperate and result in Suspension or Revocation of accreditation.

WADA will consider the overall performance of the Laboratory in making decisions regarding continued accreditation. Applicant Laboratory performance on aspects of the standards described in Section 5 (such as turn-around times; Documentation Package contents, and feedback from client organizations) may be considered in this auditing.

##### **6.4.8.1 Maintenance of accreditation**

In the event that the Laboratory has maintained satisfactory performance, WADA will recommend to the WADA Executive Committee that the Laboratory be re-accredited.

##### **6.4.8.2 Suspension of accreditation**

Whenever WADA has reason to believe that Suspension may be required and that immediate action is necessary in order to protect the interests of WADA and the Olympic movement, WADA may immediately suspend a Laboratory's accreditation. If necessary, such decision may be taken by the Chairman of the WADA Executive Committee.

Examples of actions that could result in Suspension of accreditation include:

- Suspension of ISO 17025 accreditation;
- failure to take appropriate corrective action after an unsatisfactory performance;
- lack of compliance with any of the requirements or standards listed in *WADA International Standard for Laboratories* (including Annex A. Proficiency Testing);
- failure to cooperate with WADA or the relevant Testing Authority in providing documentation;
- failure to comply with the *WADA Laboratory Code of Ethics*.

WADA may recommend a Suspension of accreditation at any time based on the results of the Proficiency Testing program.

The period and terms of Suspension shall be proportionate to the seriousness of the non-compliance(s) or lack of performance and the need to ensure accurate and reliable drug testing of *Athletes*. A period of Suspension shall be up to 6 months, during which time any non-compliance must be corrected. If the non-compliance is not corrected during the Suspension period, the Laboratory accreditation will be revoked.

In the case of a non-compliance WADA may suspend the Laboratory from performing analyses for any *Prohibited Substances*. If WADA determines that the non-compliance is limited to a class of *Prohibited Substances*, WADA may limit the suspension to analysis for the class of compounds in which the non-compliance occurred.

#### 6.4.8.3 Revocation of accreditation

The WADA Executive Committee revokes accreditation of any Laboratory accredited under these provisions if WADA determines that Revocation is necessary to ensure the full reliability and accuracy of drug tests and the accurate reporting of test results. Revocation of accreditation may be based on, but not limited to, the following considerations:

- Loss of ISO 17025 accreditation;
- Unsatisfactory performance in analyzing and reporting results of drug tests
- Unsatisfactory participation in performance evaluations or Laboratory on-site audits;
- Failure to take appropriate corrective action following an unsatisfactory performance either in *Testing* or in a proficiency test;
- A material violation of this standard or other condition imposed on the Laboratory by WADA;

- Failure to correct a lack of compliance with any of the requirements or standards listed in *WADA International Standard for Laboratories* (including Annex A: Proficiency Testing) during a Suspension period;
- Failure to cooperate with *WADA* or the relevant Testing Authority during the Suspension phase;
- A serious violation of the Code of Ethics;
- Conviction of any key personnel for any criminal offence committed that is related to the operation of the Laboratory; or
- Any other cause that materially affects the ability of the Laboratory to ensure the full reliability and accuracy of drug tests and the accurate reporting of results.

A Laboratory whose accreditation has been revoked is ineligible to perform testing of *Doping Control Samples* for any Testing Authority.

If a Laboratory whose accreditation has been revoked should seek accreditation, it shall begin the process as a new laboratory as described in Section 4.1, unless there are exceptional circumstances or justifications as determined solely by *WADA*. In the case of exceptional circumstances, *WADA* shall determine what steps shall be followed prior to granting a new accreditation.

#### 6.4.9 Notification

##### 6.4.9.1 Written Notice

When a Laboratory is suspended or *WADA* seeks to revoke accreditation, *WADA* must immediately serve the Laboratory with written notice of the Suspension or proposed Revocation by facsimile, mail, personal service, or registered or certified mail, return receipt requested. This notice shall state the following:

- 1) The reason for Suspension or proposed Revocation;
- 2) The terms of the Suspension or proposed Revocation; and
- 3) The period of Suspension.

##### 6.4.9.2 Effective Date

A Suspension is immediately effective. A proposed Revocation is effective 30 calendar days after the date on the written notice or, if review is requested, upon *WADA*'s decision to uphold the proposed Revocation. A Laboratory who has received notice that its accreditation is in the process of being revoked shall be suspended until the Revocation is made final or is rescinded by *WADA*. If *WADA* decides not to uphold the Suspension or proposed Revocation, the Suspension is terminated immediately and any proposed Revocation shall not take place.

#### 6.4.9.3 Public Notice

WADA will immediately notify all relevant national public authorities, *National Anti-Doping Organizations*, *National Olympic Committees*, International Federations, and the IOC of the name and address of any Laboratory that has had its accreditation suspended or revoked, and the name of any Laboratory that has had its Suspension lifted.

WADA will provide to any Testing Authority, upon written request, WADA's written decision which upholds or denies the Suspension or proposed Revocation.

#### 6.4.10 Re-accreditation Costs

On an annual basis, WADA will invoice the Laboratory for a portion of the costs associated with the re-accreditation process. The Laboratory shall assume the travel and accommodation expenses of the WADA representative(s) in the event of on-site inspections.

#### 6.4.11 Issue and publication of Accreditation certificate

If maintenance of accreditation is approved, the Laboratory shall receive a certificate signed by a duly authorized representative of WADA issued in recognition of such accreditation. Such certificate shall specify the name of the Laboratory and the period for which the certificate shall be valid. Certificates may be issued after the effective date, with retroactive effect.

#### 6.5 Accreditation Requirements for Satellite Facilities for Major Events

In general, the reporting time requirements for a major *Event* require that the Laboratory facility be at the location in proximity to the competition such that *Samples* can be delivered by *Event Doping Control* staff. This may require relocation of an existing Laboratory for a period of time sufficient to validate operations at the satellite facility and perform the testing for the *Event*.

In extraordinary circumstances, *Samples* may be transferred to an existing Laboratory facility. There must be agreement between the *Major Event Organization* and WADA regarding whether testing requirements such as turn-around time and the *Athlete* rights are met for in any eventuality. If the Laboratory is functioning within its regular facility, the requirements stated below with respect to facilities do not apply. The Laboratory will, however, be required to report on staffing, equipment, and *Sample* transport issues.

The Laboratory shall be responsible for providing WADA with regular updates on the progress of the testing facilities.

#### 6.5.1 Participate in an initial WADA/ISO visit/inspection

WADA may visit the Laboratory facility as soon as it is available to determine whether the facility is adequate. Expenses related to such a visit shall be at the Laboratory's expense. Particular emphasis will be placed on the adequacy of security

considerations, the physical layout of the space to ensure that adequate separation of various parts of the Laboratory are maintained, and to provide a preliminary review of other key support elements.

#### **6.5.2 Document ISO/IEC 17025 accreditation of the satellite facility**

At least one month prior to the major *Event*, the Laboratory must provide documentation that the national accrediting body has provided ISO/IEC accreditation for the satellite facility in compliance with the Application of ISO/IEC 17025 to the Analysis of *Doping Control Samples* (Section 5). WADA may require that a WADA consultant be present at the national accrediting body audit of the satellite facility. WADA's expenses associated with such audit, will be at the Laboratory's expense.

#### **6.5.3 Complete a Pre-Event Report on Facilities and Staff**

At least one (1) month prior to the *Event*, the Laboratory must report:

- List of Laboratory staff
- List of staff scientists not normally employed by the Laboratory (if required)
- Training plan for new staff scientists
- List of instrumental resources and equipment
- Procedure manual specific to the satellite facility including analytical methods
- Summary of results management process including criteria for determining positive and negative results
- Methods of reporting test results in a secure manner to the appropriate authorities

Any changes that occur prior to the *Event* should be immediately reported to WADA.

Even if the testing is to be done at the Laboratory's regular facility, the *Pre-Event* Report must be completed, particularly in regard to personnel changes and any additional equipment.

#### **6.5.4 Participate in WADA accreditation audit**

WADA may choose to perform an independent on-site audit or a document audit of the satellite facility. Should an on-site audit take place, WADA expenses related to the audit will be at the Laboratory's expense. This audit may include analysis of a set of proficiency testing samples. The full complement of staff must be in attendance. Particular emphasis will be placed on involvement of new staff members to assess their competence.

#### **6.5.5 Review the reports and correct identified non-conformities**

The Laboratory Director must address and correct any identified non-compliances. The audit report and documentation of the corrective actions must be submitted to WADA.

#### **6.5.6 Issue and publication of a temporary and limited Accreditation certificate**

Based on the documentation provided, WADA shall make a decision regarding accreditation of the Laboratory. In the event that accreditation is awarded, WADA shall issue an accreditation for the period of the *Event* and an appropriate time before and after the actual competition.

#### **6.5.7 Monitoring and assessment during the Event**

WADA may choose at its sole discretion to have an observer in the Laboratory during the *Event*. The Laboratory Director is expected to provide full cooperation to the observer.

WADA, in conjunction with the *Major Event Organization*, will submit double blind proficiency testing samples to the Laboratory.

In the event of a false positive, the Laboratory will immediately cease testing for the class of *Prohibited Substances and Methods*. The Laboratory shall apply corrective actions within 12 hours of notification of the false positive. All *Samples* analyzed prior to the false positive will be re-analyzed for the class of *Prohibited Substances and Methods* for which the non-compliance occurred. The results of the investigation and analysis will be presented to WADA within 24 hours unless otherwise agreed in writing.

In the event of a false negative, the Laboratory will be required to investigate the root cause and apply corrective actions within 24 hours of notification of the false negative result. A representative group of *Samples* in appropriate number to ensure that the risk of false negatives is minimal will be re-analyzed for the class of *Prohibited Substances and Methods* for which the non-compliance occurred. The results of the investigation and analysis will be presented to WADA within 48 hours unless otherwise agreed in writing.

### **7.0 Requirements for supporting an Adverse Analytical Finding in the Adjudication Process**

This section describes the relevant procedures to be followed where an *Athlete* challenges an *Adverse Analytical Finding* in a hearing as provided for by the *Code*.

#### **7.1 Laboratory Documentation Package**

In support of any *Adverse Analytical Finding* the Laboratory is required to provide the Laboratory Documentation Package described in detail in the Technical Document on Laboratory Documentation Packages.

The Laboratory is not required to provide any documentation not specifically included in the Laboratory Documentation Package. Therefore, the Laboratory is not required to support an *Adverse Analytical Finding* by producing, either to the Testing Authority International Standard for Laboratories

or in response to discovery requests related to the hearing, standard operating procedures, general quality management documents (e.g., ISO compliance documents) or any other documents not specifically required by Technical Document on Laboratory Documentation Packages. References in the *International Standard for Laboratories* to ISO requirements are for general quality control purposes only and have no applicability to any adjudication of any specific *Adverse Analytical Finding*.

## PART THREE: ANNEXES

### ANNEX A - WADA PROFICIENCY TESTING PROGRAM

The WADA Proficiency Testing (PT) Program is designed to evaluate Laboratory proficiency and to improve test result uniformity between Laboratories, and to provide educational opportunities for the WADA-accredited Laboratories. The purpose of the individual PT sample will determine its composition and form.

#### 1. Probationary period

The Proficiency Testing (PT) program is a part of the initial evaluation of a Laboratory seeking accreditation. In addition to providing samples as part of quarterly PT samples, the WADA will provide upon request samples from past PT rounds in order to allow the applicant Laboratory with an opportunity to evaluate its performance against the recorded performance of accredited Laboratories.

All procedures associated with the handling and testing of the PT samples by the Laboratory are, to the greatest extent possible, to be carried out in a manner identical to that applied to routine Laboratory Samples, unless otherwise specified. No effort should be made to optimize instrument (e.g., change multipliers or chromatographic columns) or method performance prior to analyzing the PT samples unless it is a scheduled maintenance activity. Methods or procedures used in routine testing should be employed.

Successful participation in 12-24 months of PT sample rounds is required before a Laboratory is eligible to be considered for accreditation. The PT samples shall occur at least quarterly and will consist of a minimum of five (5) samples per challenge. At least four (4) PT samples will contain Threshold Substances. Blank and adulterated samples may also be included.

#### 2. Maintenance/Re-accreditation period

After accreditation, Laboratories shall be challenged with at least five (5) PT samples each quarter. Each year at least two (2) samples will contain Threshold Substances. Blank and adulterated samples may be included.

All procedures associated with the handling and testing of the PT samples by the Laboratory are, to the greatest extent possible, to be carried out in a manner identical to that applied to routine Laboratory Samples, unless otherwise specified. No effort should be made to optimize instrument (e.g., change multipliers or chromatographic columns) or method performance prior to analyzing the PT samples unless it is a scheduled maintenance activity. Methods or procedures not used in routine testing should not be employed.



## 2.1 Open PT Samples

The Laboratory may be directed to analyze a PT sample for a specific *Prohibited Substance*. In general, this approach is used for educational purposes or for data gathering.

## 2.2 Blind PT Samples

The Laboratory will be aware that the sample is a PT sample, but will not be aware of the content of the sample. Performance on blind PT samples is to be at the same level as for the open or non-blind PT samples.

## 2.3 Reporting – Open and Blind Proficiency Samples

The Laboratory should report the results of open and blind PT samples to WADA in the same manner as specified for routine *Samples*. For some samples or PT sample sets, additional information may be requested from the Laboratory.

## 2.4 Double Blind Proficiency Sample

The Laboratory will receive PT sample sets which are indistinguishable from normal testing samples. The samples may consist of blank, adulterated or positive samples. These samples may be used to assess turn-around time, compliance with documentation package requirements, and other non-analytical performance criteria as well as Laboratory proficiency.

# 3. Proficiency Test Sample Composition

## 3.1 Description of the Drugs

PT samples contain those *Prohibited Substances*, *Metabolite(s)* of *Prohibited Substances*, and *Marker(s)* of *Prohibited Substances and Methods* which each accredited Laboratory must be prepared to assay in concentrations that allow detection of the analytes by commonly used screening techniques. These are generally concentrations that might be expected in the urine of drug users. For some analytes, the sample composition may consist of the parent drug as well as major *Metabolites*. The actual composition of the PT samples supplied to different Laboratories in a particular PT sample may vary but, within any annual period, all Laboratories participating are expected to have analyzed the same total set of samples.

A sample may contain more than one *Prohibited Substance*, *Metabolite(s)*, or *Marker* of a *Prohibited Substance or Method*. A PT sample will not contain more than three substances or their *Metabolite(s)*, or *Markers* of *Prohibited Substances or Methods*. It is possible that the sample will contain multiple *Metabolites* of a single substance, which would represent the presence of a single *Prohibited Substance*. All *Metabolites* detected should be reported according to the Laboratory's standard operating procedures.

## 3.2 Concentrations

PT samples may be spiked with *Prohibited Substances* and/or their *Metabolites* or may be from authentic administration studies. For Threshold Substances, the

concentration in the sample will be guided by, but not limited to, one of the following criteria:

- i) at least 20 percent above the threshold for either the initial assay or the confirmatory test, depending on which is to be evaluated;
- ii) near or below the threshold limit for special purposes. In this case, the Laboratory would be directed to analyze the Sample for a particular Prohibited Substance as part of an educational challenge and will not be considered for evaluation for the purposes of the PT program.

For Non-threshold Substances, the concentration will be guided by, but not limited to, one of the following criteria:

- i) the Prohibited Substance and/or its major Metabolite(s) will be present in quantities greater than the Minimum Required Performance Limit;
- ii) the Prohibited Substance and/or its major Metabolite(s) will be present near the limit of detection for special purposes. In this case, the Laboratory would be directed to analyze the sample for a particular Prohibited Substance as part of an educational challenge and will not be considered for evaluation for the purposes of the PT program.

These concentrations and drug types may be changed periodically in response to factors such as changes in detection technology and patterns of drug use.

Negative samples do not contain concentrations of any of the target drugs above the Minimum Required Performance Limit when analyzed by the normally used methods.

### 3.3 Blank or Adulterated Samples

PT samples include those that do not contain prohibited drugs or samples which have been deliberately adulterated by the addition of extraneous substances designed to dilute the sample, degrade the analyte or to mask the analyte during the analytical determination.

## 4. Evaluation of Proficiency Testing Results

### 4.1 Evaluation of Quantitative Results

When a quantitative determination has been reported, the results can be scored based on the true or consensus value of the sample analyzed and a standard deviation which may be set either by the group results or according to the expected precision of the measurement. The z-score is calculated using the equation

$$z = \frac{x - \hat{x}}{\delta}$$

Where x is the value found

$\hat{x}$  is the assigned value

$\delta$  is the target value for standard deviation

The target relative standard deviation will be set in such a way that an absolute z-score between two (2) and three (3) is deemed **questionable** performance. A z-score greater than three (3) is deemed **unacceptable** performance.

In addition, re-scaled sum of score (RSZ) and re-scaled sum of squared scores (RSSZ) will be calculated. While the z score gives an estimate of bias, the RSZ, by retaining the sign of the biases, will reflect consistent systematic bias. The RSSZ, by eliminating the possibility that positive and negative bias will cancel, provides another indicator of bias. The RSZ and RSSZ are calculated by the equations

$$RSZ = \sum \frac{z}{\sqrt{m}}$$

$$RSSZ = \sum \frac{z^2}{m}$$

where m is the number of tests.

#### **4.2 Probationary Period**

**4.2.1** Any false positive reported automatically disqualifies a Laboratory from further consideration for accreditation. The Laboratory will be eligible for reinstatement upon providing documentation that satisfies WADA that remedial and preventative actions have been implemented.

**4.2.2** An applicant Laboratory is to achieve an overall grade level of 90 percent for PT samples required during the probationary period, i.e., it must correctly identify and confirm 90 percent of the total drug challenges (qualitative including adulterated samples).

**4.2.3** An applicant Laboratory is to obtain satisfactory Z-scores for any quantitative results reported based on the mean of three replicate determinations. For the purposes of accreditation a quantitative result is required for threshold drugs. The relative standard deviation is to be commensurate with the validation data.

Any Laboratory that fails to achieve a satisfactory score for at least 90% of the quantitative determinations during the probationary period will be disqualified from further consideration. If the Laboratory receives fewer than 10 samples for quantitation in the year, the Laboratory may be allowed a single unsatisfactory result in the quantitative portion of the PT program during a 12 month period. The Laboratory will be eligible for reinstatement upon providing documentation that satisfies WADA that remedial and preventative actions have been implemented.

### 4.3 Maintenance and Re-Accreditation Period

**4.3.1** No false positive drug identification is acceptable for any drug and the following procedures are to be followed when dealing with such a situation:

- i) The Laboratory is immediately informed of a false positive error by the WADA.
- ii) The Laboratory is to provide the WADA with a written explanation of the reasons for the error within five (5) working days. This explanation is to include the submission of all quality control data from the batch of samples that included the false positive sample. If the error is deemed to be technical/scientific.
- iii) The WADA shall review the Laboratory's explanation promptly and decide what further action, if any, to take.
- iv) If the error is determined to be an administrative error (clerical, sample mix-up, etc), the WADA may direct the Laboratory to take corrective action to minimize the occurrence of the particular error in the future and, if there is reason to believe the error could have been systematic, may require the Laboratory to review and re-analyze previously run Samples.
- v) If the error is determined to be a technical or methodological error, the Laboratory may be required to re-test all Samples analyzed positive by the Laboratory from the time of final resolution of the error back to the time of the last satisfactory proficiency test round. A statement signed by the Laboratory Director shall document this re-testing. The Laboratory may also be required to notify all clients whose results may have been affected of the error as part of its quality management system. Depending on the type of error that caused the false positive, this retesting may be limited to one analyte, a class of Prohibited Substances or Methods, or may include any prohibited drug. The Laboratory shall immediately notify the WADA if any result on a Sample that has been reported to a client is detected as a false positive. WADA may suspend or revoke the Laboratory's accreditation. However, if the case is one of a less serious error for which effective corrections have already been made, thus reasonably assuring that the error will not occur again, the WADA may decide to take no further action.
- vi) During the time required to resolve the error, the Laboratory remains accredited but has a designation indicating that a false positive result is pending resolution. If the WADA determines that the Laboratory's accreditation must be suspended or revoked, the Laboratory's official status becomes "Suspended" or "Revoked" until the Suspension or Revocation is lifted or any process complete.

**4.3.2** An accredited Laboratory must correctly identify 100 percent of the Prohibited Substances to pass the round of PT samples. It must correctly identify and confirm 100 percent of the total PT samples (qualitative including adulterated samples).

**4.3.3** An accredited Laboratory is to obtain satisfactory Z-scores for any quantitative results reported based on the mean of three replicate determinations. For the purposes of accreditation a quantitative result is required for threshold drugs.

The relative standard deviation is to be commensurate with the validation data.

Any Laboratory that fails to achieve a satisfactory score for quantitative determinations will be deemed to have failed that sample challenge. The Laboratory must achieve a satisfactory score on 90% of the quantitative samples during the year. If the Laboratory receives fewer than 10 samples for quantitation in the year, the Laboratory may be allowed a single unsatisfactory result in the quantitative portion of the PT program during a 12 month period.

- 4.4 Laboratories failing a proficiency test round are informed immediately by WADA. Laboratories must take and report corrective action within 30 calendar days to WADA. Laboratories may otherwise be advised by WADA to take corrective action for a given reason or to change a corrective action which has previously been reported to WADA. The corrective action reported to WADA must be implemented in the routine operation of the Laboratory. Repeated failures of the same type will result in WADA requiring corrective action.

Laboratories failing two consecutive rounds of the PT scheme will be immediately suspended. The Laboratory is required to provide documentation of corrective action with 10 working days of notification of Suspension. Failure to do so will result in immediate Revocation of the accreditation. Lifting of the Suspension occurs only when corrective action has been taken and reported to the WADA. The WADA may choose, at its sole discretion, to submit additional PT samples to the Laboratory or to require that the Laboratory be re-audited, at the expense of the Laboratory after having furnished satisfactory results for another proficiency testing round.

- 4.5 WADA is to evaluate the annual performance of all accredited Laboratories.

## ANNEX B - LABORATORY CODE OF ETHICS

### 1. Confidentiality

The heads of Laboratories, their delegates and Laboratory staff shall not discuss or comment to the media on individual results prior to the completion of any adjudication without consent of the organization that supplied sample to the Laboratory and the organization that is asserting the *Adverse Analytical Finding* in adjudication.

### 2. Research

Laboratories are entitled to participate in research programs provided that the Laboratory director is satisfied with the *bona fide* nature and the programs have received proper ethical (e.g. human subjects) approval.

#### 2.1. Research in Support of *Doping Control*

The Laboratories are expected to develop a program of research and development to support the scientific foundation of *Doping Control*. This research may consist of the development of new methods or technologies, the pharmacological characterization of a new doping agent, the characterization of a masking agent or method, and other topics relevant to the field of *Doping Control*.

#### 2.2. Human subjects

The Laboratories must follow the Helsinki Accords and any applicable national standards as they relate to the involvement of human subjects in research.

Voluntary Informed consent must also be obtained from human subjects in any drug administration studies for the purpose of development of a Reference Collection or proficiency testing materials.

#### 2.3. Controlled substances

The Laboratories are expected to comply with the relevant national laws regarding the handling and storage of controlled (illegal) substances.

### 3. Testing

#### 3.1. Competitions

The Laboratories shall only accept and analyze *Samples* originating from known sources within the context of *Doping Control* programs conducted in competitions organized by national and international sports governing bodies. This includes national and international federations, *National Olympic Committees*, national associations, universities, and other similar organizations. This rule applies to Olympic and non-Olympic sports.

Laboratories should exercise due diligence to ascertain that the *samples* are collected according to the World Anti-Doping *Code International Standard* for

Testing or the International Standard for Doping Control (ISO/PAS 18873), or similar guidelines. These guidelines must include collection of Split Samples; appropriate Sample container security considerations; and formal chain-of-custody conditions.

### 3.2. **Out-of-competition**

The Laboratories shall accept Samples taken during training (or Out-of-competition) only if the following conditions are simultaneously met:

- (a) That the Samples have been collected and sealed under the conditions generally prevailing in competitions themselves as in Section 3.1 above;
- (b) If the collection is a part of an anti-doping program; and
- (c) If appropriate sanctions will follow a positive case.

Laboratories shall not accept Samples, for the purposes of either screening or identification, from commercial or other sources when the conditions in the above paragraph are not simultaneously met.

Laboratories shall not accept Samples from individual Athletes on a private basis or from individuals or organizations acting on their behalf.

These rules apply to Olympic and non-Olympic sports.

### 3.3. **Clinical or Forensic**

Occasionally the Laboratory is requested to analyze a Sample for a banned drug or endogenous substance allegedly coming from a hospitalized or ill Person in order to assist a physician in the diagnostic process. Under this circumstance, the Laboratory director must explain the pre-testing issue to the requester and agree subsequently to analyze the Sample only if a letter accompanies the Sample and explicitly certifies that the Sample is for medical diagnostic or therapeutic purposes.

The letter must also explain the medical reason for the test.

Work to aid in forensic investigations may be undertaken but due diligence should be exercised to ensure that the work is requested by an appropriate agency or body. The Laboratory should not engage in testing or expert testimony that would call into question the integrity of the individual or the scientific validity of work performed in the anti-doping program.

### 3.4. **Other Testing**

If the Laboratory accepts Samples from an entity that is not a Testing Authority recognized by the World Anti-Doping Code, it is the responsibility of the Laboratory Director to ensure that any Adverse Analytical Finding will be processed according to the Code and that the results cannot be used in any way by an Athlete or associated Person to avoid detection.

The Laboratory should not engage in testing that undermines or is detrimental to the anti-doping program of WADA. The Laboratory should not provide results that in any way suggests endorsement of products or services for Athletes or sports authorities. The Laboratory should not provide testing services in defense of an Athlete in a Doping Control adjudication.

### **3.5. Sharing of Information and Resources**

#### **3.5.1 New Substances**

The WADA-accredited Laboratories for Doping Control shall inform WADA when they detect a new or suspicious doping agent.

When possible, the Laboratories shall share information regarding the detection of potentially new or rarely detected doping agents.

#### **3.5.2 Sharing of Knowledge**

Sharing of knowledge shall consist of, but not be limited to, dissemination of information about new *Prohibited Substances and Methods* and their detection within sixty (60) days of discovery. This can occur by participation in scientific meetings, publication of results of research, sharing of specific details of methodology necessary for detection, and working with WADA to distribute information by preparation of a reference substance or biological excretion study or information regarding the chromatographic retention behaviour and mass spectra of the substance or its *Metabolites*. The Laboratory director or staff shall participate in developing standards for best practice and enhancing uniformity of testing in the WADA-accredited Laboratory system. An example of the latter would be in establishing reporting standards for determination of an *Adverse Analytical Finding*.

### **4. Conduct Detrimental to the Anti-Doping Program**

The Laboratory personnel shall not engage in conduct or activities that undermine or are detrimental to the anti-doping program of WADA, an International Federation, a *National Anti-Doping Organization*, a *National Olympic Committee*, a *Major Event Organization Committee*, or the International Olympic Committee. Such conduct could include, but is not limited to, conviction for fraud, embezzlement, perjury, etc. that would cast doubt on the integrity of the anti-doping program.

No Laboratory employee or consultant shall provide counsel, advice or information to *Athletes* or others regarding techniques or methods to mask detection of, alter metabolism of, or suppress excretion of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method* in order to avoid an *Adverse Analytical Finding*. No Laboratory staff shall assist an *Athlete* in avoiding collection of a *Sample*. This paragraph does not prohibit presentations to educate *Athletes*, students, or others concerning anti-doping programs and *Prohibited Substances* or *Methods*.



## ANNEX C - LIST OF TECHNICAL DOCUMENTS

Title	Document Number	Version Number	Effective Date
Laboratory Internal Chain of Custody	TD2003LCOC	1.2	Jan 1, 2004
Laboratory Documentation Packages	TD2003LDOC	1.3	Jan 1, 2004
Minimum Required Performance Limits for Detection of Prohibited Substances	TD2004MRPL	1.0	Feb15,2004
Identification Criteria for Qualitative Assays Incorporating Chromatography and Mass Spectrometry	TD2003IDCR	1.2	Jan 1, 2004
Reporting Norandrosterone Findings	TD2004NA	1.0	Aug13, 2004
Reporting and Evaluation Guidance for Testosterone, Epitestosterone, T/E Ratio and other Endogenous Steroids	TD2004EAAS	1.0	Aug13, 2004
Harmonization of the Method for the Identification of Epoetin Alfa and Beta (EPO) and Darbepoetin Alfa (NESP) by IEF-Double Blotting and Chemiluminescent Detection	TD2004EPO	1.0	<i>In progress</i>
Measurement of Uncertainty for Anti-Doping Analysis			<i>Future</i>
Reporting Guidance for Gas Chromatography/Combustion/ Isotope Ratio Mass Spectrometry			<i>Future</i>
Reporting Guidance for Salbutamol and other Beta-2 Agonists			<i>Future</i>



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## **ADDENDUM TO THE INTERNATIONAL STANDARD FOR LABORATORIES**

### **REQUIREMENTS FOR ANTI-DOPING ANALYSIS OF WHOLE BLOOD, PLASMA, SERUM OR OTHER BLOOD FRACTIONS.**

Several anti-doping tests have now been developed on the blood matrix, and can be applied to whole blood or blood fractions (e.g. plasma, serum) to determine doping practices in sport.

As currently established, the World Anti-Doping Code *International Standard* for Laboratories does not specifically cover procedures to handle and analyze the blood matrix in anti-doping Laboratories. Provision 5.2.4.4.1 of the *International Standard* for Laboratories refers to specific requirements for the analysis of the blood matrix to be promulgated separately.

The present document is established to complement or amend the existing *International Standard* for Laboratories, to provide ad hoc requirements to the Laboratories for handling and analyzing blood *Samples* in the context of anti-doping analysis.

The official text of the Addendum to the *International Standard* for Laboratories shall be maintained by WADA and shall be published in English and French. In the event of any conflict between the English and French versions, the English version shall prevail.

### **Specific Requirements for Whole Blood or Blood Fractions Analyses**

In any Sections that refer to urine, and are carried over into this document by reference, the terms blood, plasma, or serum shall be substituted as appropriate. Unless otherwise stated, there is no blood, plasma, or serum equivalent to the urine integrity test or data, and any reference to this should be deleted.

The following sections of Section 5 of the *International Standard* for Laboratories apply to the analysis of blood *Samples* by reference:

**5.1** and all subsections;

**5.2.1** and all subsections;

**5.2.2** and all subsections with the exception of subsections 5.2.2.5 and 5.2.2.6 which are replaced by the following:

Provisions 5.2.2.5 and 5.2.2.6 apply to plasma, serum or other blood fractions containing no blood cells. *Samples* shall be frozen on reception until analysis and as soon as practical after aliquots have been taken for analysis. The Laboratory shall retain the A and B *Samples* for a minimum of three (3) months after the Testing Authority receives a negative report. The *Samples* shall be retained frozen under appropriate conditions.

*Samples* with irregularities shall be held frozen for a minimum of three (3) months following the report to the Testing Authority.

*Samples* that consist of whole blood or blood fractions containing intact cells shall be stored at approximately 4 degree Celsius on reception and should be analyzed within 48 hours. As soon as practicable after aliquots have been taken for analysis, *Samples* should be returned to approximately 4 degree Celsius storage. The anti-doping Laboratory shall retain the A and B *Samples* with or without *Adverse Analytical Finding* for a minimum of 1 month after the Testing Authority receives the final analytical ("A" or "B" *Sample*) report.

**5.2.3** and all subsections;

**5.2.4** all subsections with the exception of subsections 5.2.4.1, 5.2.4.3.1.1, 5.2.4.2.1, 5.2.4.2.4, 5.2.4.3.1.2, 5.2.4.3.2.1, which are replaced or amended where needed by the following:

5.2.4.3.1.1 Screening and confirmation tests may be performed initially on the same aliquot of *Sample*. The test should be repeated on a fresh aliquot of the *Sample* to ensure that the initial test results are repeatable from the same *Sample* bottle.

Detection of blood transfusion relies upon the use of multiple antibodies and flow cytometry to reveal several red blood cell antigens. Consequently article 5.2.4.3.1.3 does not apply for this type of immunochemical analysis.

5.2.4.3.2.1, for "B" *Sample* confirmation in whole blood or blood fraction with blood cells only, the "B" *Sample* analysis shall be completed within 30 days of notification of an "A" *Sample Adverse Analytical Finding*.

**5.2.5** and all subsections;

**5.2.6** and all subsections with the exception of 5.2.6.4, 5.2.6.7, and 5.2.6.8.

5.3 and all subsections;

5.4 and all subsections with the exception of 5.4.4.1, 5.4.4.2.2, 5.4.4.3, 5.4.6, and 5.4.7 which are amended, where applicable, by the following:

**5.4.4.1 Selection of Methods**

Standard methods are generally not available for *Doping Control* analyses. The Laboratory shall develop, validate and document in-house methods for substances on the *Prohibited List* or their Metabolites or Markers. The methods shall be selected and validated so they are fit for the purpose.

5.4.4.3 The Laboratory should provide an estimation of the measurement uncertainty where applicable.

**5.4.6.2 Reference Collection**

A collection of *Samples* or isolates may be obtained from a biological matrix following an authentic and verifiable administration or traceable mixture of a *Prohibited Substance* or *Method*, providing that the analytical data are sufficient to justify the identity of the *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Metabolite* of a *Prohibited Substance* or *Marker* of a *Prohibited Substance* or *Method*.

**5.4.7. Assuring the quality of test results**

5.4.7.1. The performance of Laboratories for analysis on the blood matrix will be evaluated as deemed necessary by the *World Anti-Doping Agency* under the principles of the *International Standard for Laboratories* specifically applied to the blood matrix.

5.4.7.2 The Laboratory shall have in place a quality assurance system, including the submission of blind quality control samples, that challenges the entire scope of the testing process.

5.4.7.3 Analytical performance should be monitored by operating quality control schemes appropriate to the type and frequency of blood testing performed by the Laboratory.

Applicable Technical Documents for blood analysis:

Laboratory Documentation Packages.

Laboratory Internal Chain of Custody.



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Montreal, January 15<sup>th</sup>, 2007

Dear Laboratory Directors,

Following the CAS decision in the recent T/E ratio case of the cyclist I. Landaluce (TAS 2006 / A /119) having exonerated the athlete on the basis that the same technician was involved in the preparation of the A and the B samples, WADA would like to remind all the accredited laboratories of the need to follow strictly provision 5.2.4.3.2.2 of the International Standard for Laboratories in order to avoid dismissal of laboratory results in court on a procedural basis. This matter will be addressed further at the next WADA Laboratory Committee meeting.

We use this opportunity to emphasize the obligation of accredited laboratories to follow all the rules applicable to them as established in the WADA standards.

Should you require further information, please do not hesitate to contact me.

Yours truly,

A handwritten signature in black ink, appearing to read "Rabin", with a horizontal line underneath.

Dr. Olivier Rabin  
Director, Science  
World Anti-Doping Agency

## ANNEX 6

### WADA Technical Document – TD2003LDOC

Document Number:	TD2003LDOC	Version Number:	1.3
Written by:	WADA Project Team	Approved by:	
Date:	June 5, 2003	Effective Date:	January 1, 2004

#### LABORATORY DOCUMENTATION PACKAGES

The required Documentation Packages shall be provided by the Laboratory whenever required by the *International Standard* for Laboratories or in support of an *Adverse Analytical Finding* challenged by an *Athlete*. The package will be comprised of the information listed below. Each page of the package shall be numbered sequentially and the package certified to be a true copy of the original data and forms. The items listed below do not constitute a list of required flow charts, forms or documents, but instead a list of information necessary to support the analytical result. Laboratory working documents, computer printouts, and similar documents may be in the native language of the laboratory personnel. Table of contents and any flowcharts explaining the sequence of steps in the process and any other explanatory portions of the Documentation Packages should be provided in English or French, if requested.

The items listed below shall be the only information the Laboratory is required to include in the Documentation Package. Therefore, the Laboratory is not required to support an *Adverse Analytical Finding* by producing standard operating procedures, general quality management documents (e.g., ISO compliance documents) or any other documents not specifically required below.

**All Documentation Packages provided shall contain the following information:**

- Table of Contents
- List of Laboratory Staff involved in the test, including signatures and/or initials and position title(s)
- *Sample* Collection Control Form (external chain of custody form)
- Documentation of shipping and receipt of intact *Sample*
- Documentation linking *Sample* Identification Number to Laboratory Identification Number
- "A" *Sample* Bottle Laboratory Internal Chain of Custody
- Urine Integrity test results (if completed)

## WADA Technical Document – TD2003LDOC

Document Number:	TD2003LDOC	Version Number:	1.3
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Date:	June 5, 2003	Effective Date:	January 1, 2004

- Screening Test Data
  - Screening Test Description
  - Screening Aliquot Laboratory Internal Chain of Custody documentation
  - Screening Test Results on negative, positive and athlete Aliquot
  - Documentation of any deviations from the written screening procedures, if any
  - Data run in the same analytical run or used to verify instrument performance or operation during that run.  
*[For example, tuning data for the mass spectrometer; chromatographic performance verification samples, if any; and/or quality control data, if any. This does not refer to data generated at other times (e.g., validation data for the method)]*
- "A" Sample Confirmation Procedure Data
  - Confirmation Procedure Description
  - Confirmation Aliquot Laboratory Internal Chain of Custody documentation
  - Confirmation Procedure Data on negative, positive and all Athlete Aliquot(s)
  - Quantitative Data or ratio data and uncertainty estimation, if applicable
  - Documentation of any deviations from the written confirmation procedures, if any *[For example, a change in the split ratio or a dilution of the derivatized sample due to sample overload in the GC/MS; application of an additional cleanup step; or an explanation for the re-analysis of the sample with a new aliquot.]*
  - Data run in the same analytical run or used to verify instrument performance or operation during that run.  
*[For example, tuning data for the mass spectrometer; chromatographic performance verification samples, if any; and/or*

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*quality control data, if any. This does not refer to data generated at other times (e.g., validation data for the method)]*

- "A" Sample Certificate of Analysis or certified Test Report
- Documentation of identity of "B" Sample with information on opening procedure and signature of athlete, representative, or surrogate present at opening
- "B" Sample Bottle Laboratory Internal Chain of Custody
- "B" Sample Confirmation Procedure Data
  - Confirmation Procedure Description
  - Confirmation Aliquot Laboratory Internal Chain of Custody documentation
  - Confirmation Procedure Data on negative, positive and all *Athlete Aliquot(s)*
  - Data run in the same analytical run or used to verify instrument performance or operation during that run. *[For examples, see "A" sample section]*
  - Quantitative Data or ratio data and uncertainty estimation, if applicable
  - Documentation of any deviations from the written confirmation procedures, if any *[For examples, see "A" sample section]*
  - "B" Sample Certificate of Analysis or certified Test Report



# ANNEX 7



## 2005 Adverse Analytical Findings Reported by Accredited Laboratories

**Table G** Substances Identified in Each Drug Class

<b>S1.1.a. Anabolic Agents - Exogenous AAS</b>	<b>Occurrences</b>	<b>% within drug class</b>
Nandrolone	298	16.0%
Stanozolol	233	12.5%
Methandienone	56	3.0%
Methenolone	28	1.5%
Boldenone	28	1.5%
Mesterolone	11	0.6%
1-testosterone	7	0.4%
Methyltestosterone	7	0.4%
1-androstendione	7	0.4%
Drostanolone	6	0.3%
Oxandrolone	6	0.3%
Oxymetholone	5	0.3%
Trenbolone	5	0.3%
Mestanolone	4	0.2%
Dehydrochloromethyltestosterone	4	0.2%
Clostebol	2	0.1%
Danazol	2	0.1%
Methandriol	2	0.1%
delta-1-DHT	1	0.1%
<b>subtotal*</b>	<b>712</b>	

<b>S1.1.b. Anabolic Agents - Endogenous AAS<sup>1</sup></b>	<b>Occurrences</b>	<b>% within drug class</b>
Testosterone	1,132	60.7%
Prasterone (DHEA)	6	0.3%
Androsterone	5	0.3%
Androstenedione	4	0.2%
Etiocholanolone	4	0.2%
5β-androstanediol	1	0.1%
<b>subtotal*</b>	<b>1,152</b>	

<b>S.1. All Anabolic Agents</b>	<b>Occurrences</b>
<b>TOTAL*</b>	<b>1,864</b>

<sup>1</sup> Reporting of an Endogenous AAS may be due to detection of a concentration outside normal reference ranges and/or establishment of an exogenous source by GC/C/IRMS.

<b>S2. Hormones and Related Substances</b>	<b>Occurrences</b>	<b>% within drug class</b>
hCG	143	88.3%
Erythropoietin	15	9.3%
LH	3	1.9%
Darbepoetin	1	0.6%
<b>TOTAL*</b>	<b>162</b>	

\* Some adverse analytical findings correspond to multiple findings on the same athlete, including cases of longitudinal studies on testosterone.